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JAMES JOHNSON, 1886-1952

G. W. Keitt and R. W. Fulton

James Johnson, Professor of Horticulture and Plant Pathology of the University of Wisconsin, died suddenly at his home on August 4, 1952.

The youngest of 7 children of Hans and Karen Andrea Martinsen Johnson, James was born at Deerfield, Wisconsin, May 11, 1886. His parents had come to America from Norway in 1884 and settled in this predominantly Norwegian community. James was graduated from Deerfield High School in 1904. He later entered the University of Wisconsin, receiving the B. S. degree in 1909, the M. S. in Horticulture in 1911, and the Ph. D. in Plant Pathology in 1918.

A long and fruitful career in agricultural research was begun in 1909 when he started studies on the culture and diseases of tobacco as an Assistant in the Department of Horticulture. His interest in this crop probably arose from his early familiarity with tobacco culture and from its great importance in his home community. James Johnson's interest and work on diseases of tobacco were further stimulated by his contacts with Professor L. R. Jones, who came to Wisconsin in 1910 to found the Department of Plant Pathology. Throughout the succeeding years of Johnson's scientific work he continued to expand his range of interests in plant pathology, though he remained in the Department of Horticulture. He was promoted to the ranks of Instructor in 1911, Assistant Professor in 1916, Associate Professor in 1920, and Professor in 1922. He participated actively in the work of the Department of Plant Pathology, and in 1944 his title was changed to Professor of Horticulture and Plant Pathology.

Professor Johnson's research program gained support both from the University and the United States Department of Agriculture, with which he entered into a cooperative relationship in 1915 and in which during most of his career he was an agent or a collaborator.

His interests and researches covered a wide range of subjects relating to tobacco. One of his first studies was of damping-off of tobacco seedlings, in which he pioneered in developing methods for steam sterilization of tobacco seed beds. Among his other early interests were the decays and rots encountered in tobacco curing and fermentation. He determined the ranges of temperature and relative humidity that minimized these difficulties.

Yields of tobacco in Wisconsin when Professor Johnson began his work were becoming progressively lower. This he found to be due to the black root rot disease, for which he soon achieved a successful control by developing, for the first time, disease-resistant tobacco varieties. One of these, Havana 142, was released in 1922 and is still extensively grown. In his subsequent years he continually sought to improve the agronomic and disease-resistant characteristics of Wisconsin tobacco. Almost the entire acreage in Wisconsin for many years has been planted with resistant varieties that he developed.

JAMES JOHNSON
1886—1952

Together with L. R. Jones and others, Johnson pioneered in the development of the first Wisconsin soil temperature tank. One of the first research studies on soil temperature relations at Wisconsin was concerned with black root rot of tobacco. This began Johnson's lifelong interest in the relation between environment and disease.

Additional tobacco diseases occupied Professor Johnson's attention in succeeding years. He described Fusarium wilt of tobacco in 1921. Other root diseases interested him, and he made basic studies on the brown root rot disease. In the early 1920's the tobacco wildfire disease appeared in Wisconsin. Transmission and control of this disease were the subjects of several research papers. Characteristically, he sought fundamental factors influencing infection and the occurrence of this disease. He described the relation between wildfire infection and the flooding of intercellular spaces of tobacco leaves by water. Factors affecting water congestion of leaf tissue by root pressure were investigated with the realization that this was a mechanism by which environment affected disease. These studies were later expanded and the importance of water congestion to infection by various pathogenic organisms was demonstrated.

Professor Johnson's scientific work on tobacco was

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by no means limited to pathology. The introduction of black root rot resistant varieties permitted continuous cropping of tobacco, and he was quick to see the need for adequate methods of soil management and fertilization. An extensive series of field experiments in the 1930's and early 1940's brought out the importance of proper nutrition to quality in tobacco, in particular to the ability of the cured leaf to hold a glow after being ignited. Many cultural practices currently followed with Wisconsin tobacco are outgrowths of these researches. He also inaugurated and supervised a tobacco seed certification program which was enthusiastically accepted by the growers and dealers.

Contacts with the mosaic disease of tobacco inevitably aroused Professor Johnson's interest and curiosity early in his career. Pioneering work was being done on virus diseases in the 1920's, and to this he contributed a large share. In determining the effect of temperature on the black root rot disease, he had been interested in developing controlled air temperature chambers. These were turned to the study of tobacco and potato virus diseases, and the masking of symptoms by high temperatures was described in 1921. A few years later he described mild strains of tobacco mosaic virus obtained by keeping infected plants at high temperatures for long periods. This was one of the earliest indications of the existence of virus strains.

His interest in plant viruses expanded in many directions in the decade from 1920 to 1930. He described the potato latent or X-virus, then present in all American tubers, a discovery which helped clarify the nature of virus complexes in this and other crops. He developed methods of separating and characterizing viruses as distinct entities on the basis of their tolerance to heat, dilution, and aging, and by differential host reactions. This quickly led to his recognition of the need for an adequate nomenclature and classification for plant viruses. His pioneering in this field resulted in his being designated as an organizer with H. M. Quanjer, of an international committee on plant virus nomenclature in connection with the 6th International Botanical Congress at Amsterdam. Almost continuously from that time he served on committees concerned with this problem. He devised and championed the use of a numbering system, recognizing it as a practical expedient that could readily be supplanted when the state of knowledge might warrant a system of classification based on natural affinities.

Despite his wide range of research interests, Professor Johnson was never content merely to explore immediate practical possibilities. In any problem he was quick to look for fundamental principles. Work on control of tobacco mosaic showed that the normally stable virus lost its activity rather readily when in contact with some supposedly innocuous substances. He subsequently expanded these observations into a series of investigations, seeking to determine the true nature of virus inactivation. In more recent work he studied strains of tobacco mosaic virus, with special reference to their origin and separation. He was keenly interested in electron microscopy of viruses.

Though he served continuously at the University of Wisconsin, Professor Johnson's activities extended widely. His cooperative relations with the United States Department of Agriculture led to periods of study in Washington and to consultation in many states. He became a recognized authority on a wide variety of tobacco problems and his counsel was sought by research institutions and industrial organizations as well as by growers.

He directed the research of many graduate students who worked on problems concerning tobacco or virus diseases. One of these, the late Dr. Ismé Hoggan, left a bequest in support of a collection of literature concerning virus diseases. With this aid, and in part with his private resources, Professor Johnson assembled a remarkably complete collection of virus literature. Interest in the history of virology led to special efforts to obtain early papers of historical importance, several of which he translated for Phytopathological Classics. In accordance with his wishes his virus library was presented to the University of Wisconsin by his family. It is now part of the library of the Department of Plant Pathology, where it will be maintained as the Johnson-Hoggan Memorial Collection of virus literature. Continued contribution of reprints from those who publish on virus disease will provide a fitting tribute, which will be available for use by any who are interested in virus diseases.

Professor Johnson was a member of the American Association for the Advancement of Science, the American Phytopathological Society, Sigma Xi, and the American Association of University Professors.

On September 16, 1913, he was married to Miss Dorothy Ella Perrin of Little Rock, Arkansas, who made a gracious and hospitable home and aided him generously in his life's work. Besides his wife, he is survived by their 2 children, Mrs. Walter Heller and David P. Johnson, 2 sisters, 2 brothers, and 5 grandchildren.

James Johnson was deeply devoted to his science and to the service of his fellow man. He was quick to sense both basic and applied research problems in his field and was energetic and resourceful in his efforts to solve them. He was always ready to share his enthusiasm and knowledge, and many experienced the cordial hospitality of his laboratory and his home. He keenly enjoyed argument and discussion of scientific topics. In his busy life he still found time for many recreational activities. He was an ardent devotee of the faculty bowling league for many years and took great interest in the University intercollegiate athletic contests. Though his health was failing for many months he continued until his last day the work to which he was devoted. He will long be remembered for his substantial contributions to the science of plant pathology and for his high qualities as a man.

Professor Johnson was the author or coauthor of some 127 scientific papers, which are listed in the Johnson-Hoggan Memorial Collection.

INTRODUCTION TO THE SYMPOSIUM ON GENETICS OF PHYTOPATHOGENIC FUNGI IN RELATION TO BASIC PROBLEMS OF INFECTIOUS DISEASE [1]

G. W. Keitt

Genetics of microorganisms is a rapidly developing field that is shedding much light on many fundamental biological problems. Important among these are basic problems of infectious disease.

Several symposia concerning the genetics of microorganisms have been held. Especially pertinent to the field we are treating today were the Ottawa symposium on The Genetics of Pathogenic Organisms (7) in 1938, the Cold Spring Harbor symposium on Heredity and Variation in Microorganisms (6) in 1946, and the Chicago symposium on Genetics of Microorganisms[2] in 1947.

At the first of these symposia, the need for research on the genetics of pathogenic organisms was comprehensively set forth by Stakman (8). He pointed out that the problem of genetics of microorganisms is essentially the problem of their variation and that knowledge of the nature and extent of variability of pathogens is essential to the understanding of many of the most important theoretical and practical problems of disease.

The present symposium needs little further introduction. It was arranged jointly by The Mycological Society of America and The American Phytopathological Society through a committee consisting of S. J. P. Chilton, J. J. Christensen, W. L. Gordon, W. C. Snyder, and G. W. Keitt, Chairman. Because of limitations of time and lack of financial resources, it seemed best to restrict its field to Genetics of Phytopathogenic Fungi in Relation to Basic Problems of Infectious Disease.

In the years that have elapsed since the Ottawa symposium, great progress has been made in genetic and related studies of microorganisms. Genetics of bacteria (5) and of viruses (2, 4) have achieved scientific respectability and have been admitted to the genetic family circle. Still more important to the subject of the present symposium, great advances have been made in genetics of fungi. Outstanding among these has been the development of so-called biochemical genetics (1, 3), in which genetic controls of biochemical reactions furnish keys to fundamental knowledge of the mechanism of life processes. Far from being the laggard it was a decade and a half ago, microbial genetics is now rapidly moving into the vanguard of genetic and biological progress.

Of all the groups of plants and animals, perhaps no other is so rich as the fungi in forms with favorable adaptations for genetic studies in relation to basic biological problems. Those of us who work with plant diseases are fortunate in dealing with pathogenic fungi that present unique opportunity for study, not only of their genetics and the genetic control of their nutritional processes, but also of these fields in relation to many of the most fundamental problems of infectious disease. Examples of some of the more immediate and important aspects of these problems are the nature and extent of variation in pathogens, basic factors that govern the development of epidemics, the nature of parasitism and of disease resistance, the mechanisms of disease reactions between pathogens and suscepts, the development and maintenance of disease-resistant plants, and the possible development of races of pathogens resistant to chemicals used for disease control. Examples of other fundamental biological problems with important implications for pathology are the nature and inheritance of sex and the basic mechanisms of inheritance.

The advances of recent years have placed in our hands powerful new tools of genetics and biochemistry that make possible new approaches to these basic problems. Although this symposium is necessarily limited in scope, let us hope that it will be an occasion for taking stock of progress already made and will serve as a stimulus to new and fertile researches that will make good use of the rich materials and ingenious methods that are now available.

Department of Plant Pathology
University of Wisconsin
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[1] Invitational paper presented September 7, 1953, at the Symposium on Genetics of Phytopathogenic Fungi in Relation to Basic Problems of Infectious Disease, arranged jointly by The Mycological Society of America and The American Phytopathological Society at the 20th Annual Meeting of the former and the 45th of the latter at Madison, Wisconsin.

[2] Joint symposium on Genetics of Microorganisms of The National Research Council and The American Society of Naturalists, sponsored by The American Phytopathological Society, The Mycological Society of America, and The Mycological Section of The Botanical Society of America, Chicago, Illinois, December 29, 1947 (papers published individually at the author's initiative).

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SPECIES CONCEPT, GENETICS, AND PATHOGENICITY IN HYPOMYCES SOLANI [1]

William C. Snyder and H. N. Hansen

SUMMARY

Many so-called species and varieties of Hypomyces, and of Fusarium, are merely segments of 1 large, rather well-defined ubiquitous species, *H. solani*. Of the many and diverse clones which in the aggregate compose the species *H. solani*, most are saprophytic on organic debris, and relatively few have been grouped into pathogenic forms on the basis of host preference. Compatibility heterothallism is a result of sterility and interfertility factors (+ and −) which are allelomorphic, inherit independently, and which are in no way related to sex although frequently referred to as such. There are only 2 basic sexes, male and female, and these sexes are not allelomorphic but complementary, and inherit independently of one another. Hermaphroditism is probably the general rule throughout the lower haploid organisms and possibly also in higher organisms. By mutation and through segregation pathogens continually originate. These may be new races of a previously existing pathogen or arise from fungi supposed to be only saprophytes. In either case their survival is apt to depend on the presence of the right host and the right environment at the time and place of the new origination.

Hypomyces solani Rke. & Berth. em. Snyd. & Hans. (18) is an Ascomycete with fleshy red, or white, perithecia and hyaline, 2-celled, ovoid ascospores. In its conidial or haploid vegetative phase it is a Fusarium with a diversity of genetically and biologically different clones. This vegetative stage normally produces sickle-shaped macroconidia, ovoid microconidia, and terminal and intercalary chlamydospores. Thus, *H. solani* in its complete state possesses 4 kinds of spores, (Fig. 1) all haploid, and all capable of reproducing the thallus on which they may be borne. Only the ascospores are derived from a sexual fusion and reduction division of the nuclei. These spores are shot from the asci and are the sole air-borne spores of the species. The other 3 spore forms are soil- and water-borne, but may be seed-borne.

H. solani has attracted the attention of man principally because of its potent capacity for pathogenesis. However, only a small percentage of the clones, which in the aggregate comprise the species *H. solani*, are known to be pathogenic, and these only to a few crops such as pea, bean, potato and cucurbits. The diseases incited by them occur widely over the world, often destructively.

H. solani is one of the common species of the fungi. All its clones, whether pathogenic or not, are active agents in the decay of organic refuse everywhere. Yet, it is only now, after the bringing together of its scattered components, each of which previously had been considered a separate species, that we can begin to grasp the over-all species picture of *H. solani* as it occurs in nature.

Species concept in H. solani.—What is the concept of the species exemplified here, and why are we concerned with the concept? The answer to the latter question is a practical one. When a plant pathologist applies a species name to an economic fungus he expects to be able to recognize it and to have others recognize it by its description. For him and for the grower, correct identification of pathogens is important since food crops or large investments often are at stake. The pathologist can not tolerate a situation in which one and the same fungus is identified differently each time it is isolated, nor where pathogens are not distinguished from saprophytes. Thus, the pathologist is concerned with taxonomy and nomenclature because he has to use them.

Our concept of the species *H. solani* is essentially one of morphologic similarity, arrived at experimentally through an exploration of the limits of variability.

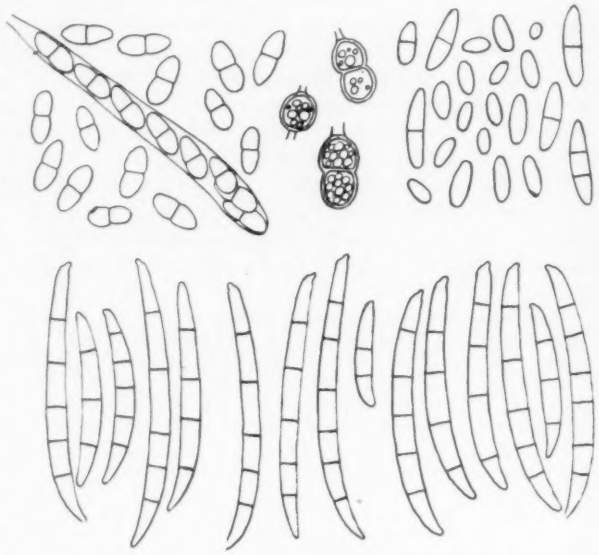

FIG. 1. The 4 spore forms of *Hypomyces solani:* macroconidia, microconidia, chlamydospores, and ascospores.

[1] Invitational paper presented September 7, 1953, at the Symposium on Genetics of Phytopathogenic Fungi in Relation to Basic Problems of Infectious Disease, arranged jointly by The Mycological Society of America and The American Phytopathological Society at the 20th Annual Meeting of the former and the 45th of the latter at Madison, Wisconsin.

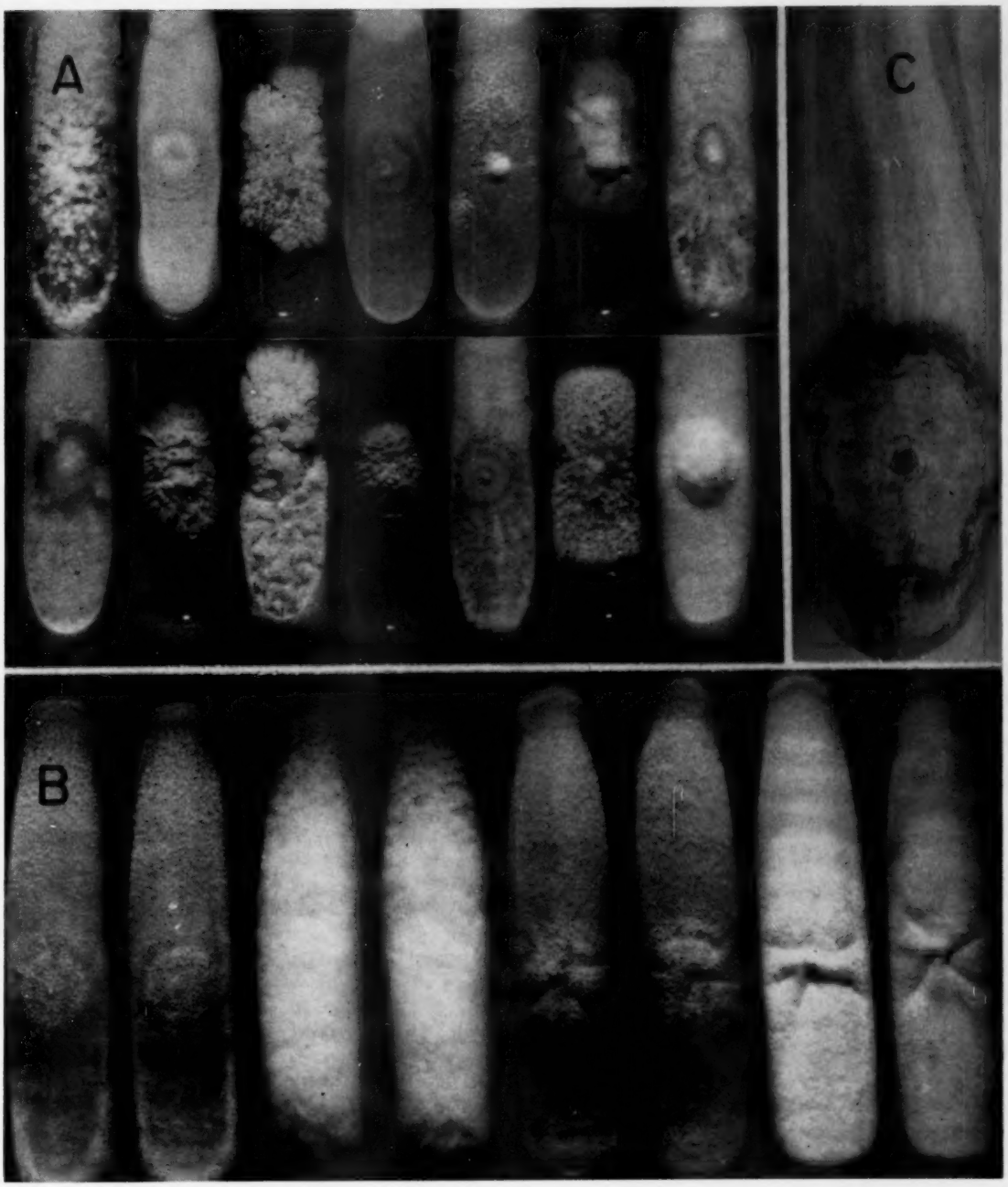

FIG. 2. *Hypomyces solani* f. *cucurbitae*. A) Representative clonal variations resulting from crossing the first 2 cultures at top left of figure. B) Eight single ascospore cultures derived from a single ascus of the cross female × male; the paired cultures from left to right are hermaphrodites, neuters, females, males. C) Sporulation on naturally infected fruit of Cocozelle squash; seed invasion by the fungus may follow.

For example, in the heterothallic form pathogenic to cucurbits (Fig. 2 C), *H. solani* f. *cucurbitae* Snyd. & Hans., the progeny of a single perithecium were assembled, and to these were added the progenies of their matings, and their mutants. The result was a wide assortment of clones which differed morphologically and biologically in many ways. This is the usual experience when any serious study is attempted on the variability of an organism. An example of the degree of variability is shown in (Fig. 2 A, B). Had these

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clones been picked up in nature, individually, and compared, they probably would have been called different species. But knowing that they all were derived from 1 perithecium, they were studied to find out not so much the characters in which they differed but those in which they were similar. It was found that the shape of the macroconidium, not its size nor number of septa but the sausage-like shape, was common to all clones and therefore was an important character upon which to tie all of the clones together into 1 species. Supplementary characters, found in the same way, include the microconidial shape, and the presence of terminal and intercalary chlamydospores.

When other Fusaria of known or suspected relationship, or from other perithecia, were compared with the range of clones obtained in *H. solani* f. *cucurbitae*, it was soon shown that these, too, fitted well into the same morphological species pattern as far as these particular characters were concerned.

Here, then, we have only 2 alternatives. Either we must treat these different clones as distinct species, or varieties, or we must recognize them for what they are —the natural variations of 1 large but rather well-defined species.

It should be emphasized here that we are not dealing merely with laboratory mutants. These clones appear in nature and are recoverable there as such. This is more than the acknowledgement of variation in a fungus, it is the use of that knowledge in fungus classification, in the delimitation of a species.

Thus, over a period of years and after examining several hundred clones, it is now evident to us that the biologically different pathogens of pea, bean, potato, squash, etc., and the various saprophytic clones found on all kinds of crop debris, and the homothallic and heterothallic and sterile forms, many of which previously were treated as different species in the sections Martiella and Ventricosum, belong morphologically to 1 species, *F. solani* (Mart.) App. & Wr. em. Snyd. & Hans., with the perfect stage in *H. solani*.

Because it is important for the pathologist to distinguish between pathogenic and saprophytic clones, those found by test to be selectively pathogenic to a particular host are further designated as formae or as races, involving a trinomial system of nomenclature. No way is known for distinguishing between pathogenic and saprophytic clones other than by actual test on living plants.

This concept of a species is based on the precept that any fungus should be identifiable to species, when all its morphological stages are present, irrespective of its substrate or its ecology. A modified version of the species and nomenclature concepts used here has been adopted recently by Fischer and Shaw (4) for the smuts. However, these authors compromise between a morphological and host basis in that they require that the host family be known before the smut species can be set apart morphologically.

Genetics of H. solani.—Genetical studies have provided not only a sounder basis for speciation, but also an increased understanding of the biology of the species. Perhaps the greatest contribution of genetics in this species has been the clarification of the phenomena of heterothallism and sex inheritance.

It has been shown in *H. solani* f. *cucurbitae*, for example, that there are 2 kinds of heterothallism, both of which are physiological phenomena (14). One, which may be called compatibility heterothallism, involves intrasterility, interfertility factors, usually referred to as plus (+) and minus (−). These factors, which are allelomorphic, and which inherit in a 1:1 ratio, are completely independent of the factors for sex. These factors determine whether or not mating between sexually perfect clones may take place. A (+) and a (−) hermaphrodite will mate, but 2 (+) hermaphrodites or 2 (−) hermaphrodites will not. Normally a fungus showing compatibility heterothallism is hermaphroditic, self-sterile and 50 per cent interfertile as has been shown for *Puccinia graminis tritici* Erickss. & E. Henn. (1), *H. solani* (2), *Venturia inaequalis* (Cke.) Wint. (13) and *Endoconidiophora fagacearum* Bretz (9).

The other kind of heterothallism may be referred to as sex heterothallism, which involves a loss of sex factors in thalli which originally were sexually perfect. Expressions of this phenomenon are found in fungi which are referred to generally as being homothallic in nature, such as *Glomerella cingulata* (Stoneman) Spauld. & v. Schr., *Ceratostomella fimbriata* (Ell. & Hals.) Elliott, and certain clones of *H. solani*. In the homothallic condition, these fungi are self-fertile hermaphrodites, and compatibility factors if contained in the nuclei, are then not evident. It has been observed in these and also in heterothallic fungi (3, 6, 8, 15) that mutant clones may develop as mycelial types, giving expression to the Dual Phenomenon (5). Particular clones of *Ceratostomella fimbriata*, or *Hypomyces solani*, although self-sterile can function in matings, but only as males, i.e., they are unable to develop perithecial primordia but their vegetative cells may serve as spermatia. Mutants also occur which resemble in appearance the normal self-fertile parent but which are self-sterile. They, also, may function in matings, but only as females, i.e., they develop perithecial primordia but the vegetative cells are unable to function as spermatia. These 2 kinds of self-sterile mutants, derived from self-fertile parents, will mate to produce normal perithecia and ascospores. The mutants, then, display a heterothallic condition brought about independently of compatibility, by the loss of the female factor (ability to produce perithecial fundaments) in the first case and loss of the male factor in the second. In *Glomerella cingulata* the effect of different genes upon sterility, interfertility, and upon the matings of clones has received much study (20).

A striking and unexpected inheritance of the sex factors is revealed in an analysis of the mating of a unisexual female by a unisexual male (7, 10). Not only are males and females recoverable in the progeny but also neuters and hermaphrodites. This is signifi-

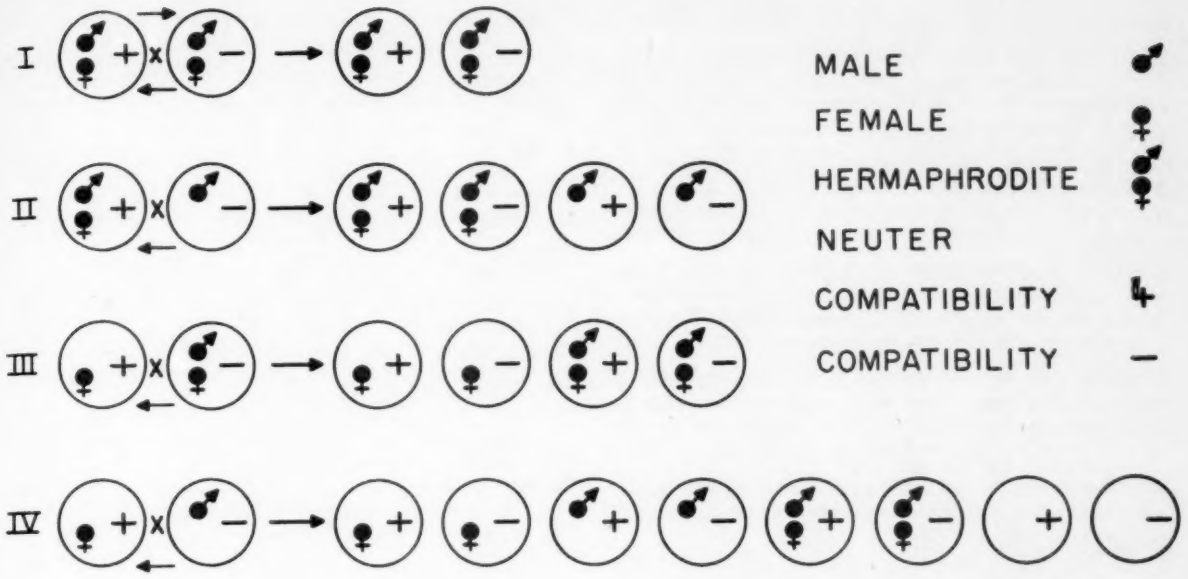

FIG. 3. Inheritance of factors for compatibility, and of factors for male, and female, in *Hypomyces solani* f. *cucurbitae*. Each circle represents a haploid nucleus. I) Compatibility heterothallism. The sexually perfect, haploid nuclei (hermaphroditic) are self-sterile, 50 per cent interfertile, i.e., mating is consummated only if opposite compatibility types (+) and (−) are brought together. In this case fertilization may take place in either direction, the progeny consist of hermaphrodites only, and (+) and (−) inherit independently in a 1:1 ratio. II, III) Compatibility heterothallism combined with sex heterothallism. Fertilization takes place in 1 direction only. Although it would appear that hermaphrodites and unisexuals inherit independently in a 1:1 ratio also, it is shown in IV that this is not so. IV) Like in II and III, except that it is clear here that the basic sex factors, male and female, are independent, not alleles, and therefore that hermaphroditism in II and III does not inherit as a unit character.

cant from the sex standpoint since it is evidence that the male and female factors are not allelomorphic but complementary. Here, also, is a mechanism in the fungus to restore in nature the original hermaphroditic and homothallic conditions when these are lost through mutation. Furthermore, here is an instance of the sudden origin de novo, of sexless, imperfect clones from a sexually perfect fungus.

In *H. solani* f. *cucurbitae* both compatibility and sex heterothallism may occur simultaneously. A summary of the foregoing phenomena of the 2 heterothallisms, and sex, are illustrated in Fig. 3.

These revelations of the biologic nature of *H. solani*, and of other fungi, are examples of the kind of facts which genetical studies may provide and which the plant pathologist needs in order to understand the potentialities and limitations of variability and of propagation, the origin of new forms or races, and the losses in fertility in such infectious pathogens as those which incite oak wilt, apple scab, smuts and rusts, and various root rot diseases. These revelations also provide needed facts for an informed, practical taxonomy and nomenclature.

PATHOGENICITY IN H. SOLANI.—Comparative studies on the pathogenicity of different clones of fungi of known genetic constitution have been made for the smuts and rusts (11, 19), and for the pathogens of apple scab (12), and squash root rot (16). These studies have reflected the kinds of differences in pathogenicity, and virulence, that should be expected from genetically diverse individuals, or clones.

There is increasing evidence that new clones, and old ones, are continually appearing and reappearing in nature in any 1 fungus, some more pathogenic than others, and that ecology, including man's cropping practises, and the degree of clonal pathogenicity in the fungus largely determine which clones may survive.

As more accurate means are developed for measuring pathogenicity and for maintaining clonal lines of host and parasite, more will be revealed as to the nature of the fungus-host relationship. Examples of this and an indication of what is to come is found in the races just established for *Venturia inaequalis* (17) and the current discovery of a second race of *H. solani* f. *cucurbitae* on the cucurbits.

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GENETICS AND EVOLUTION OF HETEROTHALLISM IN GLOMERELLA [1]

H. E. Wheeler

One of the characteristics of living organisms is their ability to undergo spontaneous, heritable changes. In plant pathogens, this ability poses for plant pathologists the never-ending problem of combating the new forms which are constantly arising. If the pathologist and plant breeder are to gain the initiative in this struggle, prophylactic measures designed to prevent the occurrence of new races of pathogens must be substituted for the passive defenses employed at present. The possibility of developing such prophylactic measures seems to be dependent on the acquisition of a better knowledge of the nature of spontaneous mutations and the mechanisms responsible for their occurrence. Although it is highly improbable that immediate solutions for these basic problems will be forthcoming, an encouraging feature of the situation is that mutant forms offer powerful tools for analysis of biological processes. This has been clearly demonstrated by the remarkable results achieved in the field of biochemical genetics with induced mutants of *Neurospora* and other fungi which have been used to investigate steps in the biosynthesis of various amino acids and vitamins. It would appear that similar methods might be profitably used to investigate other biological processes, including parasitism. With this in mind, mutant forms of *Glomerella* have been used in an attempt to analyze the process of sexual reproduction in this fungus.

Glomerella cingulata (Stonem.) S. & v. S., the incitant of bitter rot of apples and similar diseases of other fruits, is a notoriously variable species. Although its instability in culture has been cited as a major disadvantage for studies of the nature of parasitic disease, this same feature makes it well suited for basic studies of the nature of variability in fungi (20, 24). The variety of self-fertile and self-sterile types which occur and which readily undergo cross-fertilization, make this organism also well adapted to basic studies of sexuality and the evolution of sexual differentiation in fungi (4, 7, 19, 20, 24).

Interest in the nature of sexuality in members of the genus *Glomerella* was aroused by Edgerton, whose pioneer studies (8, 9) constituted the first report of sexual differentiation in the ascomycetes. The 2 types described by Edgerton in 1914 each possessed a low

[1] Invitational paper presented September 7, 1953, at the Symposium on Genetics of Phytopathogenic Fungi in Relation to Basic Problems of Infectious Disease, arranged jointly by The Mycological Society of America and The American Phytopathological Society at the 20th Annual Meeting of the former and the 45th of the latter at Madison, Wisconsin.

Based on studies carried out in part under the terms of Contract No. W-7405-eng-26 for the Atomic Energy Commission at Oak Ridge and Contract No. AT-(40-1)-1083 at Louisiana State University and supported in part by a grant from the Committee for Research on Problems of Sex of the National Research Council.

degree of self-fertility, otherwise they closely resembled the opposite mating types subsequently described in *Neurospora* and various other fungi. Twenty years later, Hüttig, who apparently was unaware of Edgerton's work, published the results of his study of sexuality in *Glomerella* isolated from tomato (11).

Hüttig, who interpreted his results on the basis of Hartmann's theory of relative sexuality, described self-fertile types which he called hermaphrodites as well as sterile types which he called males. It is interesting to note, as Dodge has pointed out (6), that some of the cultures which Hüttig termed male were capable of producing perithecial fundaments. Andes' paper in 1941 marked the beginning of an extensive series of genetic, cytological, and physiological investigations designed to determine the nature of variability in members of this genus (1-5, 10, 13, 14, 15-25). Although these studies will not be reviewed, it should be noted that the results which have been obtained indicate that variation in this fungus is explicable on the basis of gene mutation and selection (5, 21, 24). Furthermore, it has been demonstrated clearly that inheritance in *Glomerella* is governed by the same fundamental laws which are operative in other organisms (3, 4, 22, 24).

Before proceeding with this discussion, it will be necessary to arrive at a working definition of 2 terms —*heterothallism* and *homothallism*. From a genetic point of view, heterothallism may be defined as a condition which requires the bringing together of 2 genetically differentiated nuclei for the completion of the sexual process. Insofar as analysis of genes controlling the sexual process is concerned, it is immaterial whether these genetically different nuclei occur in separate thalli, as a heterokaryon in a single thallus or a single spore, or even whether one of them arises from the other by mutation, as has been reported in the case of *Chromocrea spinulosa* (17). Homothallism, defined in similar terms, is a condition in which the sexual process is completed in the absence of genetic differentiation. Such a definition implies that in homothallic organisms the nuclei which undergo karyogamy and meiosis are genetically identical. Accumulated genetic and cytological evidence indicates that this is the case in homothallic cultures of *Glomerella* (4, 18, 24, 25).

In the course of studies of variation in *Glomerella*, a large number of homothallic and heterothallic forms were isolated. Some of the homothallic forms were highly self-fertile; others exhibited varying degrees of self-sterility. Whereas some of the heterothallic forms produced perithecial fundaments, others completely lacked this ability. It soon became apparent that the number of different types which could be isolated was limited only by the industry of the investigator and that the types found could not be classified into 2 or 4 mating types or compatibility groups as has been done with many other fungi. More disconcerting than this great variety of types was the behavior of certain sterile forms. Grown alone, these lacked the ability to produce perithecial fundaments and in crosses made by spermatizing techniques they behaved like the so-called "pure" or "unisexual" males described in other fungi. However, in crosses made by pairing cultures, these sterile forms were stimulated to produce perithecia and to undergo self-fertilization (18, 24).

These unusual findings led to a detailed genetic and cytological study of the sexual process in this organism. The results, some of which have been published (13, 18, 24, 25), indicated that genes at many different loci were involved, each gene pair affecting a specific step in the sexual process. Most of the mutant alleles at these loci acted as partial or complete genetic blocks, interrupting the sexual process in much the same way that mutant genes have been shown to block steps in the synthesis of required growth factors in *Neurospora*.

The effects of various genes which block different steps in the sexual process are shown diagrammatically in Fig. 1. For this discussion, the sexual process has been arbitrarily divided into 4 steps: 1) perithecial initiation, 2) plasmogamy, 3) karyogamy or nuclear fusion, and 4) meiosis or reduction division. It should be emphasized that these steps are not single events; for example, plasmogamy involves copulation, disintegration of the cell walls separating the copulating elements, nuclear transfer, and the establishment of the dicaryon (18). In Fig. 1, the heavy line of broken arrows extending across the figure is intended to symbolize the sexual process in *Glomerella* which occupies a position somewhere between typical homothallism and true heterothallism. The vertical lines, pointing downward and terminating in genetic symbols, represent blocks at various steps in the process—broken lines indicating partial genetic blocks; solid lines, complete blocks. Cultures which carry the latter are, of course, completely heterothallic. The vertical lines pointing upward indicate genes which repair these blocks, giving rise to highly self-fertile homothallic types. The system of genetic symbolism used in Fig. 1 with wild-type alleles indicated by superscript plus signs and mutant alleles by superscript numerals has been previously described (24).

In *Glomerella*, the first step in the sexual process, perithecial initiation, occurs in the form of lateral outgrowths of 2 adjacent cells of a hypha. These outgrowths, or initials, form 2 coils of cells; the outer coil eventually produces the perithecial wall and the inner becomes an ascogonial coil (18). In wild-type cultures, perithecial initiation is partially blocked as the result of an interaction of 2 wild-type genes, A^+ and B^+ (24). This partial block is completely removed if either of the 2 mutant alleles, A^2 or B^1, is substituted at 1 of these loci. A third allele at the A locus, A^1, acts as a complete block and cultures which carry this gene do not form perithecial initials.

Most of the mutant genes studied block plasmogamy, the second step in the sexual process (Fig. 1). Three of the genes which block this step (arg^1, bi^1, th^1) also control requirements for growth factors (arginine,

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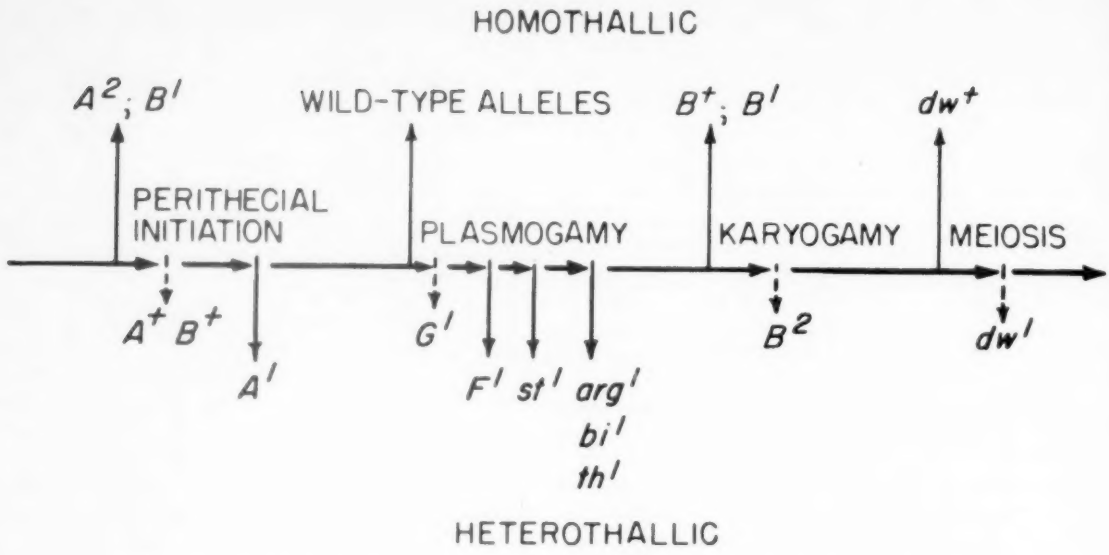

FIG. 1. Genetic blocks in the sexual process in *Glomerella*. See text for further explanation.

biotin, and thiamine). These 3 genes are nonallelic; they are shown together in the diagram merely to conserve space. Cultures that carry any of the genes which block plasmogamy produce an abundance of perithecial initials, but these never develop. They remain as small sclerotia-like masses barely visible to the unaided eye. Illustrations and detailed descriptions of the effects of F^1 and st^1 have been published (24).

Only 1 gene, B^2, has been found which affects karyogamy. Cultures which carry B^2 produce perithecia which develop normally up to and including the formation of croziers. Unpublished cytological studies have shown that in such cultures nuclear disintegration occurs just prior to or during karyogamy in more than 98 per cent of the asci. Hence, perithecia produced by these cultures, though normal in appearance, are almost completely sterile.

The effects of the mutant gene dw^1 (dwarf), which partially blocks meiosis and also controls the production of dwarf ascospores, have been described in detail elsewhere (23). In cultures which carry dw^1, 70–80 per cent of the asci abort as the result of nuclear disintegrations which occur during the meiotic divisions.

All the genetic blocks shown in Fig. 1 are repaired in matings unless both members of the mated pair carry the same mutant gene. For example, matings of A^1 cultures, in which perithecial initiation is inhibited, and F^1 cultures, in which plasmogamy is blocked, result in the formation of a dense ridge of fertile perithecia between the mated cultures (24). These cultures, both of which are completely self-sterile, behave as opposite mating or compatibility types and, in *Glomerella*, the number of such types seems to be virtually unlimited. In many respects such mating reactions resemble a phenomenon which Lilly and Barnett have termed "mutualistic symbiosis in regard to vitamins" (12). These workers have shown that if *Phycomyces blakesleeanus*, which requires thiamine for growth, and *Sordaria fimicola*, which requires biotin, are planted a short distance apart on a medium containing neither vitamin, only sparse growth occurs until the cultures meet, at which time a great stimulation in the growth of both organisms occurs. This is explained as the result of the synthesis by each culture of the specific factor which the opposing culture requires for growth. Mating reactions in *Glomerella* can be explained on a similar basis, if the logical assumption is made that genes which affect the sexual process in this fungus do so by controlling the synthesis of specific chemical substances required for completion of the various steps. In the case cited, A^1 cultures would be deficient for some substance required for perithecial initiation while in F^1 cultures the synthesis of a substance necessary for plasmogamy is blocked. In matings, each culture supplies the specific factor required for the other; hence sexual reproduction occurs when the 2 cultures meet.

These remarks can perhaps best be summarized by drawing an analogy between homothallic organisms and organisms which are nutritionally independent; i.e., organisms capable of growing normally on a medium containing only a carbon source and essential mineral elements. In a sense, both types of organisms are totipotent. Any change in the growth requirements of a nutritionally independent organism must obviously be in the direction of dependence and any change in the sexual potency of a homothallic organism will of necessity be in the direction of self-infertility. Nutritional dependent forms may arise as the result of gene

changes which affect any 1 of the many steps involved in the synthesis of various growth factors. In a similar way, heterothallic forms of *Glomerella* may arise from gene changes which affect any 1 of many steps in the sexual process. If the change results in a complete genetic block, neither the growth process nor the sexual process will be completed unless the block is repaired. Genetic blocks resulting in nutritional deficiencies can be repaired by reverse mutations, by forming heterokaryons, provided that 1 component is capable of synthesizing the required factor, or by adding the required factor to the growth medium,. Genetic blocks in the sexual process of *Glomerella* can also be repaired by reverse mutation and by forming heterokaryons if the dikaryotic system is considered to represent a special type of heterokaryosis. Here the analogy breaks down since the chemical substances presumed to be required for completion of various steps in the sexual process have not been isolated. However, evidence of the existence of such substances has been obtained (15, 18) and it would seem that this aspect of the problem offers a fertile field for future investigations.

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RECENT STUDIES OF WHEAT STEM RUST IN RELATION TO BREEDING RESISTANT VARIETIES [1]

E. C. Stakman [2]

The wheat stem rust situation was relatively stable in North America from 1938 to 1949, inclusive, because only races 17, 19, 38, and 56 were prevalent enough to be important, and many varieties of hard red spring wheat and some durums were almost immune from them.

The spectacular and catastrophic increase in prevalence and geographic distribution of race 15B of *Puccinia graminis tritici* in 1950, however, changed the situation abruptly, necessitated intensified research, and made it possible to expand knowledge regarding the composition and behavior of wheat stem rust. Studies since 1950 have not elucidated new principles but have reinforced known principles and emphasized the need for applying them in practical procedures. Not only has the complexity of the rust been more fully revealed, but the increased complexity of interrelationships between new biotypes of rust and new varieties of wheat has created new problems that require solution.

Several generalizations have found additional justification: 1) There now are innumerable biotypes of *P. graminis tritici*, including many that were produced recently; 2) The identification of an adequate sample of the increased number of biotypes and their grouping into races is difficult but necessary; 3) New biotypes are continually being produced by hybridization, probably by mutation, and possibly by other means; 4) New and dangerous biotypes may become widely and destructively prevalent in North America in 1 or 2 crop seasons; 5) Old, inconspicuous, and apparently innocuous biotypes or races may quickly become prevalent and destructive on new wheat varieties; 6) The reaction of some wheat varieties to some biotypes of rust may vary very widely with environmental conditions; 7) Relatively few wheat varieties that have been adequately tested are highly resistant to all races of rust under all environmental conditions; 8) Morphological and other characters of some varieties of wheat promise to be useful in reducing the damage caused by rust; 9) Despite disappointments there has been progress in reducing damage caused by rust; 10) Rust is dynamic; therefore concepts and procedures must be modified as new rust situations arise and new information becomes available.

The number of biotypes of *P. graminis tritici* appears to be comparable with that in *Ustilago zeae*, *Helminthosporium sativum*, and many other plant pathogenic fungi that can be grown on artificial media where diversity of cultural and physiologic characters can be studied readily. At Minnesota, at least 15,000 biotypes of *U. zeae* and at least 1000 of *H. sativum* have been isolated and studied, and there seems to be no visible limit to the number. Nor does there seem to be a visible limit in *P. graminis tritici*, although it is harder to recognize biotypes because of inability to grow rusts on artificial media. It is imperative, however, to learn as much as possible about individual biotypes and to group them into races in an attempt to develop a feasible and meaningful system for identification and designation.

That there are difficulties in identifying and classifying the numerous biotypes of wheat stem rust is apparent to any one with an adequate concept of the dynamics of an astronomically numerous and genetically diverse population of a microorganism that reproduces both sexually and asexually and can be grown only on living plants.

It would be extremely difficult to study precisely an adequate sample of the 70 billion aeciospores on a single barberry bush or the 50,000 billion urediospores on an acre of moderately rusted wheat. The difficulty is particularly great in studying the genetically diverse populations of spores on or near barberry bushes, where several races and biotypes often can be isolated from a single sample and where 40 or more have been isolated from relatively few samples of rusted material.

Not only is the sampling necessarily limited, but the 13 standard differential varieties now used for identifying races are no longer adequate for distinguishing all the biotypes in the samples. Therefore the approximately 275 known races and biotypes are probably only a small percentage of those in existence. How many biotypes could be recognized if it were possible to study the effects of 10,000 samples of rust on 15,000 varieties of wheat, 6,000 varieties of barley, and several hundred species and clones of wild grasses under an adequate range of environmental conditions? Even if such extensive studies were practicable, the population of biotypes would probably have changed

[1] Invitational paper presented September 7, 1953, at the Symposium on Genetics of Phytopathogenic Fungi in Relation to Basic Problems of Infectious Disease, arranged jointly by The Mycological Society of America and The American Phytopathological Society at the 20th Annual Meeting of the former and the 45th of the latter at Madison, Wisconsin.

The data regarding changes in prevalence of rust races and certain other data were obtained in the physiologic race surveys made cooperatively by the U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station. Annual results of these surveys have been issued for the years 1939-1952 inclusive. For 1939 through 1942, see 522 and 522A to C in the Bureau (Entomology and Plant Quarantine) E-series; for 1943, 1944, 1945-49, and 1950, see unnumbered Bureau publications in the Physiologic Races series.

[2] Thanks are due Dr. Helen Hart, Dr. Donald M. Stewart, and Mr. E. B. Hayden for some unpublished information; to Hasni Mohamed and Syed B. Ali, graduate students at the University of Minnesota, for unpublished data regarding races 49, 139, and 59.

before the study was finished. Obviously, then, it is essential to find out to what extent varieties of wheat and biotypes of rust can be grouped for practical purposes.

The first problem in devising a useful system for identifying and grouping biotypes of rust is to find suitable differential varieties. The 12 varieties originally selected by Stakman and Levine (10) from several hundred varieties of *Triticum* spp. that were then important in wheat production and improvement in North America were fairly adequate for about 3 decades. It was possible to predict that certain resistant varieties might become susceptible if certain rust races increased in prevalence. Thus race 3 was known to be a menace to Kanred before this variety became appreciably rusted in the field; it was postulated that race 11 might increase and attack durums; and it was known that certain races could attack Ceres under some conditions. Race 15B, distinguished from race 15 by a thirteenth differential, was known to be a menace to varieties deriving their resistance from Hope, H44, and Vernal emmer more than 10 years before it finally flared up in 1950. It was known also that varieties that owed their resistance to certain Kenya wheats could be attacked heavily by race 15B at high temperatures, but the first indications that races 49 and 139 could attack them over a wide range of conditions came from field observations.

The extent of knowledge regarding biotypes of rust depends on the number of biologic indicators—differential varieties—that can be found, and on the physical facilities and manpower available for testing them adequately under different environmental conditions. Theoretically, a race is a biotype or a group of closely related biotypes that can be distinguished from other groups of biotypes for practical or scientific purposes. In reality, what appears to be a race comprising 1 or several closely related biotypes on one set of differentials, may actually comprise biotypes that differ greatly on other differentials.

Race 15 may be taken as an example. This race was first identified in 1918, and all the 12 differential varieties except Khapli were susceptible. There seemed to be differences in degree of virulence between some isolates, but a comparative study failed to reveal them clearly. An isolate in Japan, however, was distinctly weaker and was therefore designated as 15A. In 1939 an isolate was found that was more virulent than 15 and 15A on Kota, but the difference was only in degree. A search was therefore made for a variety on which the infection type might be distinctly different. Several were fairly reliable, but the new variety, Lee, was finally selected because it was resistant to some isolates and susceptible to others. Those to which it was resistant were designated as 15 and those to which it was susceptible as 15B.

Races 15 and 15B were never abundant between 1939 and 1950; consequently there were relatively few isolates for study. In 1950, however, 316 isolates of 15B were identified in the United States. Of these, 17 seemed somewhat different in virulence and search was therefore made by Rosendo Postigo (7) for differential varieties that might distinguish them clearly. Of 99 varieties of wheat tested, 10 proved to be differentials for distinguishing 8 biotypes; and 11 varieties of barley made it possible to distinguish 6 additional ones. Thus 14 of the 17 isolates could be clearly distinguished by the use of 21 additional differential varieties.

As another example, 3 isolates of 15B from Nebraska seemed slightly different but could not be distinguished with certainty on the 13 standard differentials and several new varieties. They, however, were distinctly different on 2 new hybrids, as shown by the infection types indicated below.

Isolate	Hybrid 1	Hybrid 2
Gordon, Nebraska	O;	O;
Prague, Nebraska	X	3+
Venice, Nebraska	3	O;

The infection types are described by Stakman et al (10, p. 10). For convenience, they are given here in abbreviated form.

(O) Immune—No uredia, but sometimes there are necrotic flecks, designated by semicolon, thus, O;
(1) Very resistant—Minute uredia in relatively small necrotic areas.
(2) Moderately resistant—Uredia small to medium in more or less distinct green islands.
(3) Moderately susceptible—Uredia medium in size; chlorosis but not perceptible necrosis.
(4) Very susceptible—Uredia large; chlorosis but not necrosis.
(X) Heterogeneous—Uredia variable but intergrading.

The symbol ^{c} indicates chlorosis, and ^{n} necrosis. These symbols are necessary because occasionally infection types do not agree completely with those described above. In such cases the size of pustules is indicated in ascending series from 1–4, with notation as to the degree of chlorosis or necrosis. Plus and minus signs are used to indicate deviation from the average pustule size within a given infection type; ± indicates variability between the upper and lower limits of the type.

Hybrid 1 is (Renown × Marroqui) × (Aguilera × Kenya 324); Hybrid 2 is Kenya 324 × Mentana Sel. 12c, a sister selection to the variety Kentana. Both were produced by N. E. Borlaug in Mexico and obviously were not available as differentials before they were produced a few years ago.

Relatively small differences in infection types on the standard differentials may sometimes be reliable clues to genetic differences and to important differences in virulence. In 1952 almost all the 746 isolates of 15B identified in the United States, of which 1 from Iowa was representative, produced small but distinct flecks and occasionally very minute type 1— pustules on Khapli emmer. An isolate from barberry in Virginia produced type 2— and 1 from wheat in Oklahoma produced type 1+ to 3—. On Kentana, a new variety, the infection types were 1++, 3±, and 4±, respectively, a roughly proportional increase in infection type. Four isolates of race 11 also show how Khapli is

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an indicator of differences that are even less apparent on the variety Lerma but more apparent on 2 others, as shown below (8).

Isolate	Variety and Infection Type			
	Khapli	Lerma	Newthatch	Frontana × Thatcher 2854
Wisconsin	O;	4	4	4
Oklahoma	1=	4	3++	O;
Mississippi	2—	4—	0	O;
Minnesota	1++,3++[n]	4—	4	O;,2

Races 17, 21, 34, 38, 48, 59, 139 and certain others are known to comprise biotypes also.

The terms biotype and race are not and cannot be applied consistently until more is learned about the detailed genetics of the rust and of varieties used as differentials. Nevertheless there still is utility in the concept that races within the variety *tritici* should be distinguished by differences in major rust reaction classes and biotypes or sub-races by differences within these classes. Thus, Khapli is resistant to the isolates of 15B from Iowa, Virginia, and Oklahoma, but the differences in degree of resistance are characteristic and consistent enough to justify the conclusion that they are different biotypes but not great enough to justify considering them as distinct races. On Kentana, however, the difference between the Iowa isolate and the other 2 is great enough to justify considering them as distinct races. The addition of Lee wheat to the differentials only separates those biotypes to which it is susceptible from those to which it is resistant, but the addition of the 2 hybrids and of Kentana permits still further separation. New varieties have been tested extensively for their possible value as additional differentials in the past, but only Lee proved useful. It is now evident that many more will be useful. They are now being added, and, as still other varieties are produced and tested, the appropriate ones must be added also. The problem is simply to devise a good system, but obviously the problem is not simple.

The wide variability in infection types produced by some races and biotypes on some varieties is a further complication. As an example, Marquis and Kota are recorded in the key (10) as susceptible to race 49 and resistant to race 139. Race 49 was formerly very abundant; consequently hundreds of isolates had been studied under many conditions. But it was found only rarely during more than a decade, and race 139 never has been abundant in the United States. In 1952, however, both races became alarmingly prevalent in Mexico, especially on certain varieties with Kenya parentage, and the increase continued in 1953. The infection types produced by the 2 races in the spring and early summer agreed exactly with those given in the table of infection types (10). Subsequently, however, many isolates were very difficult to identify, as they appeared to be intermediates between the 2 races or mixtures of the 2, and in midsummer most appeared to be race 49. The situation was very confusing, but was clarified by Hasni Mohamed, who showed that race 49 produces type 4 pustules on Marquis and Kota at all temperatures tried. Race 139, on the other hand produces perfect type 2— pustules up to about 75°F. and type 4 at 85°, where at least some isolates are practically indistinguishable from race 49. Obviously, then, identifications must be made below 75° to distinguish between the 2 races. Race 59 also produces type 2— infection on Marquis at moderate temperatures and type 4 at 85° or higher. In this case, however, Syed B. Ali has shown that there always is some necrosis near the center of the pustule. He has also shown that different biotypes of race 59 are affected differently by temperature. It is now evident that controlled temperature facilities are essential in distinguishing many races or biotypes.

That many new biotypes result from recombinations in the sexual stage on barberry has often been emphasized. Race 56, which first became epidemic on the previously resistant variety Ceres in 1935, and race 15B, which became epidemic on previously resistant varieties of bread wheats and durums in 1950, were found originally on or adjacent to barberry bushes. Races 7 and 8 of *P. graminis avenae* also were known only in barberry areas for a number of years before they finally spread to other areas (11, 12).

Knowledge regarding the importance of sexual recombinations in the production and perpetuation of races is derived from controlled experiments in selfing and crossing races and from comparative studies of races on and near barberry bushes and in non-barberry areas.

From 1938 to 1949, inclusive, 4 races, 17, 19, 38, and 56 comprised more than 90 per cent of all uredial isolates in the United States, but as many as 48 races and biotypes were isolated in a single season from aecia and from uredia produced adjacent to barberry bushes. There were 10 times as many biotypes on or near barberry as in many non-barberry areas.

From 1950 to 1953, inclusive, the number of races isolated from aecial material continued to be far greater than from uredia, in proportion to the number of collections identified. This is clear from the following summary, in which the numerator is the number of races and the denominator the number of collections from which they were obtained. In 1951, 1952, and 1953 the figures for aecial collections include also some races that were collected adjacent to barberry bushes only.

	Uredial—r/col.	*Aecial*—r/col.
1950	19/810 = 1:43	30/ 34 = 1:1.1
1951	12/641 = 1:53	26/ 79 = 1:3
1952	16/933 = 1:58	33/ 89 = 1:2.7
1953	9/794 = 1:88	32/106 = 1:3.3

The importance of mutation and the possible role of hyphal fusions in production of biotypes and races

is imperfectly known and needs further investigation. The older data on mutation are summarized by Johnson and Newton (6), but little is known about the possibilities of heterocaryosis.

Regardless of the other ways in which new biotypes may arise, the major changes in rust situations in North America during the past 3 decades have been due to races produced on the barberry. This is true of races 56 and 15B of *P. graminis tritici* and races 7 and 8 of *P. graminis avenae*, which persisted by means of the aecial stage for a number of years before they became established in Southern United States and Mexico, where they can persist in the uredial stage (11, 12). Race 56 quickly established itself in Northern Mexico but required about a decade to establish itself thoroughly in Central Mexico (13). Race 15B spread faster.

Race 15B was first found on barberry near Fort Dodge, Iowa, in 1939. Since 1950 it has extended its range over virtually all the principal wheat-growing areas of North America, although it is not yet well established in the Pacific Northwest and has not been found in California.

In 9 of the 20 years prior to 1950, race 15 was not isolated at all from uredial collections in the United States, and in 10 of the other years the percentage prevalence ranged from 0.1 per cent to 0.6 per cent. In 1940 it comprised 1.1 per cent of all uredial isolates and 5 per cent of aecial isolates. Uredial isolates were obtained from Michigan, Minnesota, Missouri, Nebraska, Ohio, Oklahoma, South Dakota, Texas, and Wisconsin. Aecial isolates were from Iowa, Kansas, Michigan, North Dakota, Ohio, Pennsylvania, South Dakota, and Wisconsin. As a good differential was still being sought to distinguish between 15 and 15B, it is not known definitely how many of these isolates were 15B, but there is good evidence that many of them were.

From 1941 to 1949, inclusive, the only uredial isolates of race 15 obtained were from Minnesota in 1942, Wisconsin in 1943 (both 15 and 15B[3]) and Idaho in 1949. But it was obtained from aecia each year except 1943, when only 17 aecial collections were identified. Race 15 appeared menacing in 1941, as it was isolated from aecia collected in Michigan, Minnesota, Virginia, West Virginia, and Wisconsin and again in 1948 when it was obtained from aecia in Illinois, Ohio, Michigan, Pennsylvania, and Virginia; the latter 3 were 15B and the other 2 probably but not certainly were 15B also.

There was reason for apprehension because race 15, most of which subsequent to 1939 appeared by the criteria then available to be the virulent 15B or its equivalent, was isolated from aecial material every year except 1 from 1934 to 1949, inclusive. During this period it was collected from barberry in 16 states, including Maine, Pennsylvania, West Virginia, Ohio, Michigan, Indiana, Illinois, Wisconsin, Iowa, Minnesota, North Dakota, South Dakota, Nebraska, Colorado, Kansas and Missouri. That the rust could spread considerable distances from barberry to grains and grasses was shown in 1940, when uredial isolates were obtained from 9 states, including Oklahoma and Texas. But, even so, there was no premonition that 1950 would be the fateful year, as only 3 collections were obtained in 1949, all on or near barberry.

The annual percentage prevalence of uredial isolates of 15B in the United States for 1949 to 1953 has been 0.1, 27, 40, 58, and 63 per cent. The career of race 7 of *P. graminis avenae* parallels that of race 15B. It was isolated from or near barberry bushes in the Northeast for 7 successive years before it also "flared up" in 1950. The history of these 2 races and of certain others shows that virulent races of stem rust may be formed repeatedly by sexual recombinations and may suddenly "explode" when meteorologic and crop conditions are optimum for wide dissemination and rapid development.

All the reasons for fluctuations in prevalence of races are not known. Obviously, however, the varieties of wheat grown are sometimes a determining factor. One of the clearest examples of the role of wheat varieties in the increase of races is furnished by recent experience in Mexico, as summarized partly by Gibler, Narvaez, and Enciso (2). The variety Supremo and certain others were rust-resistant prior to 1951 when they were severely rusted by race 15B. They were replaced by Kentana, which derived resistance, at least at moderate temperatures, from a selection of Kenya. But Kentana and some lines of Kenya proved to be very susceptible to race 139, which was first isolated from aecia in the United States in 1932. This race never became abundant either in the United States or Mexico, nor is it yet abundant in the United States. But it increased rapidly on certain Kenya derivatives in Mexico in 1952, comprising 10.7 per cent of uredial isolates in that year and 13 per cent in 1953. In the United States, however, where Kenya derivatives are not yet grown commonly, the percentages in 1952 and 1953 were 0.8 and 0.7, per cent respectively. Race 49 appears to be increasing in Mexico also, and for similar reasons.

The resistance of certain varieties of wheat to certain biotypes of rust can vary within wide limits. For many years it appeared that the effect of races on varieties was relatively constant at different temperatures. Recent investigations indicate that this actually is true of many of the older race-variety combinations. In other combinations, however, there may be a change from a very high degree of resistance to complete susceptibility with increasing temperature (4); other factors and combinations of factors, many unknown, may cause great variability also. A good example is the effect of certain biotypes of race 15B on Kenya 58 and K117A. At temperatures below about 85°, these varieties may be almost immune, with infection type 0; or 1. Above 85°, however, they may be susceptible

[3] Identified too late for inclusion in mimeographed summary for 1943.

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(Unpublished results of Helen Hart and E. C. Stakman). Races 49 and 139, on the other hand, can produce type 4 infection at all temperatures that permit rust development on these varieties (5).

It is clear, therefore, that studies must be made of the interaction of rust biotype, wheat variety, and environmental factors. As it is difficult to control conditions in the field, more greenhouse space with controlled temperatures is needed in testing and breeding varieties.

It is increasingly apparent that relatively few varieties of wheat are resistant to all races of rust under all environmental conditions. The most universally resistant variety in North America has been Khapli emmer, which has never been heavily attacked by rust in the field, although grown in a great many rust nurseries since 1918. Moreover, Khapli is 1 of the differentials for determination of races and has never been completely susceptible to any of the collections of stem rust from North America.

Nevertheless, Khapli is known to be completely susceptible to some races of rust and, under some conditions, is at least moderately susceptible to certain biotypes of races 11 and 15B. Although not tested so long as Khapli, Kenya 338AC has been conspicuous also for general high resistance.

Whether it will be possible to combine genes for specific resistance to all present and future races of rust remains to be seen. Obviously it depends upon the genes for resistance in wheat and on the genes for virulence in *Puccinia graminis*. At present there can be only scientific agnosticism regarding this question. Basic researches obviously are needed to determine the genetic potentialities for resistance in wheat and the genetic potentialities for virulence in rust. Unfortunately the urgent demand for practical results in the past has made it difficult to make such basic researches on an adequate scale, but they are essential to an adequate understanding of the rust problem and to its solution.

The conclusion seems to be justified that certain varieties, although not resistant to all races of rust, have a certain degree of tolerance, apparently due to structural and functional characters which protect them somewhat against infection and subsequent development of rust. Hart and Stakman (3, 4, 9) have summarized some of these characters, and recent observations in rust nurseries where there were very heavy epidemics certainly indicate that a number of varieties, of which Webster is an example, may have many small pustules without being injured as much as many other varieties. A thorough study of the characters that enable varieties to tolerate rust, of their combinability with other similar characters and other desired agronomic characters is needed. The suggestion of Borlaug (1) regarding the production of "composite varieties" also deserves thorough trial.

Despite the disappointment in maintaining resistant varieties because of the sudden increase of 15B in 1950, and of certain other races in certain other areas subsequently, there has been progress in controlling rust. Because of barberry eradication and the use of resistant varieties, the number of races decreased in most of North America until it appeared during the period 1938–1949 that the race situation had become fairly well stabilized. But it is again complex and puzzling because many of the biotypes and races produced in areas where barberries still remain have become widely disseminated since 1950. This has become evident from the physiologic race surveys in the United States and Mexico. In 1953, as an example, 26 races were isolated from uredia in the United States, many near barberries, and 17 from uredial collections in Mexico. Some of these races, notably 14, 24, 29, 48, 49, and 139, have long been known in barberry areas, but there are indications that they are now becoming more generally prevalent, and the last 2 definitely have increased in prevalence in Mexico.

There has been progress also in incorporating more resistance or tolerance especially into certain of the hard red spring wheats, which despite the epidemic in the hard red spring wheat region in 1953 were not nearly so severely damaged by rust as certain of the varieties that they replaced and as the durums, such as Carleton and Stewart. Although bread wheat varieties such as Mida, Rival, Regent, Renown, Newthatch, and others can be severely injured by rust, it appears that they must be exposed to infection for a longer time and under more favorable conditions than many of the other varieties that they replaced. A direct comparison can be made between damage from stem rust to durums and to bread wheats in the Dakotas and Minnesota in 1953. The percentage losses are given below.

	Durums	Bread wheats
Minnesota	75	10
North Dakota	65	30
South Dakota	80	35

The characters that enabled the bread wheat varieties to escape, resist, or tolerate rust are not known and should be studied. Early maturity obviously is one, but there are still others which are imperfectly understood and require detailed study.

Finally, it should be realized that there is great value in producing varieties that resist rust, even if only in some places and in some years. In the present state of our knowledge it seems improbable that a good, universally resistant variety can be produced quickly. But losses certainly have been reduced in many areas and in many years by the use of improved varieties.

Concepts regarding the dynamics of rust must be broadened and deepened by extensive and intensive investigations. The rust situation has changed radically since 1950. Pathologists and breeders anticipated the possible appearance of new and virulent races, including specifically 15B. They even tried to produce varieties resistant to 15B, as it seemed to be the most

generally virulent of all known races. It obviously was difficult to develop varieties resistant to biotypes of 15B, or of any other race, which either were not in existance prior to 1950 or were present in such minute amounts as to escape detection. Even if all these dangerous biotypes had been known, it would have been unwise to inoculate rust nurseries with them and thus run the risk of helping them to become established.

Recent events and studies lead inevitably to the conclusion that there must be continuous investigation in the foreseeable future, that methods and materials must be adapted to changed conditions, and that changes in the rust situation must be anticipated as much as possible by research. More manpower and more physical facilities, including adequate greenhouse space with facilities for controlling temperature and other environmental conditions are essential. Concepts must be modified with changing conditions and increased knowledge. The fight against rust must be carried on continuously on a broad front. Barberry eradication in certain areas; breeding for resistance, earliness, and tolerance; and the search for more effective chemicals must be combined in an attempt to devise a combination of methods for at least preventing devastating attacks of one of the most insidious, variable, and destructive of all plant pathogenic fungi.

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GENETIC PHENOMENA IN THE SMUT FUNGI AS RELATED TO THE DYNAMICS OF THE SPECIES [1]

C. S. Holton [2]

SUMMARY

The basic characteristics of the smut fungi are controlled by genetic factors. Relationships among these factors are directly related to variability in the species. Factors governing such characters as culture type, spore morphology, gross morphology of the diseased host, and pathogenicity are inherited independently. Segregation and recombination of these factors produces new morphologic and pathogenic types. Unquestionably the interaction of these genetic phenomena is directly related to the highly dynamic nature of the smut species, both from the standpoint of their morphology and infectious nature.

INTRODUCTION.—Systematic studies on genetic relationships in the smut fungi apparently began after Kniep (27) demonstrated that sporidial fusion in *Ustilago violaceae* (Pers.) Rouss. occurred only between certain sporidia. Such fusions had long been observed in the smut fungi and earlier studies on nuclear phenomena related to the fusion process had been reported. The significance of sporidial fusions, however, had been oppositely interpreted on the one hand as a vegetative process and on the other as a sexual process. Kniep's demonstration that the fusion process was a specific reaction of certain sporidia to each other apparently clinched the argument for proponents of the sexual phenomenon concept. Subsequently the ramifications of studies on sex relationships in the smut fungi have led to studies on their genetic relationships, with particular reference to the origin of species and races by hybridization and mutation.

In further extensive studies on compatibility between species, as measured by sporidial fusions, Kniep (28) refers to the fusion process as hybridization. By this interpretation he was able to report hybridization between otherwise quite unrelated species, such as *Ustilago longissima* (Schlecht.) Meyen with *U. hordei* (Pers.) Lagerh., *U. nuda* (Jens.) Rostr., and *U. perennans* Rostr. Structure of the external membrane was an important indication of relationship, those with reticulate spores being opposed to those with smooth or slightly punctate spores, no fusions occurring between any such sporidial combinations. In other studies on *U. longissima*, Bauch (3, 4, 5) observed different degrees of compatibility as exhibited by certain fused sporidia which produced no germ tubes and others which did. Under natural conditions different spore layers in the same plant were of the same genotype whereas infected plants standing next to each other sometimes contained genotypically different fungus material, thus emphasizing the implications of genetic relationships in this species.

Experimentally produced hybrids between races of *Ustilago violaceae* were studied by Goldschmidt (13), who found that differences in pathogenicity were controlled by single Mendelian genes. Heritability of chlamydospore characters, nature of spore germination, and sporidial production was demonstrated by Kammerling (25) in studies with hybrids between *U. longissima* and its variety *macrospora*. Thus, this pioneer work on hybridization and genetic studies in these smuts was the basis for subsequent extensive studies of genetic phenomena in other smut fungi. This discussion deals with the smuts of sorghum, oats, barley, and wheat.

GENETICS OF SORGHUM SMUTS.—Basic consideration of genetic factors in the sorghum smuts was initiated by Rodenhiser (34, 35), who demonstrated heterothallism in *Sphacelotheca sorghi* (Link) Clint. and *S. cruenta* (Kühn) Potter and observed 2 sex-compatibility groups. Genetic factors were found to govern such characters as sex, sorus type, color of peridium, culture type, and pathogenicity. All these factors were inherited independently of one another. In general, on sorghum varieties susceptible to one parent and resistant to the other, both the intra- and inter-species hybrids were intermediate in virulence. On other varieties the inter-species hybrids were less virulent than either parent while the intra-species hybrids were more virulent than the parent races.

With reference to species dynamics the heritability of morphology and color of the peridia was important. The *S. cruenta* sorus-type was dominant. In segregating generations both parent types were recovered and also an intermediate type, distinctly different from both parents in morphology and color of peridia. Color factors in culture colonies were also inherited in a manner which yielded types clearly different from the parent colonies. Isenbeck (23) likewise reported the development of new culture types from hybrids between opposite color types in *Sphacelotheca sorghi*,

[1] Invitational paper presented September 7, 1953, at the Symposium on Genetics of Phytopathogenic Fungi in Relation to Basic Problems of Infectious Disease, arranged jointly by The Mycological Society of America and The American Phytopathological Society at the 20th Annual Meeting of the former and the 45th of the latter at Madison, Wisconsin.

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and Tyler (39) reported similar observations from studies with the same species.

Apparently successful inducement of hybridization between genera was reported by Tyler and Shumway (40), who crossed *Sphacelotheca sorghi* and *Sorosporium reilianum* and by Vaheeduddin (42, 43, 44), who crossed *Sphacelotheca cruenta* and *Sorosporium reilianum*. In these crosses also it was evident that many characters of these species are governed by genetic factors and that new types arise from the hybrid populations. In the first case the hybrid sori were somewhat intermediate, resembling each parent in some respects. Hybrid spores, though intermediate, were more like the *Sorosporium* parent but germinated with promycelia and sporidia resembling the *Sphacelotheca* parent. In the latter cross (*Sorosporium reilianum* × *Sphacelotheca cruenta*) the hybrid spores were intermediate in size but germinated with promycelia and sporidia much larger than either parent, suggesting heterosis. In morphology the hybrid sori differed from both parents and in some cases resembled those of *Tolyposporium filiferum* Busse. Peridial color was either brown or gray. Both colors are controlled by genetic factors and are inherited independently of sex factors on a simple multiple factor basis for brown and gray, respectively.

Hybrids between biotypes of *Sphacelotheca sorghi* gave rise to a previously unknown pathogenic race (43) and Laskaris (29) observed lysis (promycelial disintegration) in certain hybrid chlamydospores of this species and concluded that the tendency to lyse was controlled by genetic factors.

In the non-sorghum species *Sorosporium syntherismae* (Pk.) Farl. and *Sphacelotheca panici-miliacei* (Pers.) Bubak, Martin (30) observed that such characters as sorus type, spore morphology, rate of spore germination, and pathogenicity were controlled by genetic factors and inherited independently. New biotypes of both species were produced by hybridization and mutation. A mutant line of *S. syntherismae* with buff sori and smooth spores was reported by Johnson et al (24).

GENETICS OF BARLEY SMUTS.—The relationship between genetic factors and variability in the barley smuts was first indicated by Biedenkopf's (9) recognition of *Ustilago medians* as an intermediate type between *U. hordei* (Pers.) K. & S. and *U. nuda* (Jens.) K. & S. In its loose smut type and echinulate spores *U. medians* resembled *U. nuda*, whereas it resembled *U. hordei* in the production of sporidia and seedling infection. These characteristics, which are known to be governed by genetic factors in other smuts, suggested that *U. medians* originated from a hybrid between *U. hordei* and *U. nuda*. A similar intermediate type was reported by Vanderwalle (45), and Tapke (38) described *U. nigra* Tapke, which resembled *U. medians* in all essential respects. Ruttle (36) speculated on the possible hybrid or mutational origin of 5 intermediate types comprising a complete range of gradients between the *U. hordei* and *U. nuda* types. Moore and Allison (32) found that *U. medians* was widely prevalent along with the other 2 species, suggesting hybridization between *U. hordei* and *U. nuda*. Kniep's (28) earlier observation of fusion between sporidia of *U. hordei* and promycelial cells of *U. nuda* would seem to support this speculation. However, such fusions did not cause infection of the host and to date no demonstrated case of hybridization between these species has been reported. On the other hand, several cases of hybridization between *U. hordei* and *U. medians* (*U. nigra*) have been reported.

Ruttle (36) crossed an intermediate type (not designated by name) with *U. hordei* and the F_1 resembled the intermediate parent. In hybrids between *U. hordei* and *U. medians*, Allison (1, 2) observed dominance of the echinulate character of *U. medians* and reported independent segregation of factors for sex, pathogenicity, head type, and spore characters. Bever (8) reported that *U. nigra* and *U. hordei* hybridized readily but was unable to observe segregation ratios of the various genetic factors.

Albinism is relatively common in the barley smuts. Moore and Allison (32) reported an ablino strain which was intermediate between loose and covered smut and had smooth spores which germinated like *U. hordei*. It was compatible with both *U. hordei* and *U. medians*. Bever (8) obtained a nonpathogenic buff smut of barley from a cross between *U. hordei* and *U. nigra* which resembled *U. hordei* except for sorus color. The spores were smooth, hyaline, and intermediate in size, and germination resembled that of *U. nigra*.

Although sporidial fusions occurred in the buff smut, no infection of the host could be obtained, suggesting a difference in factors for sex and pathogenicity. Mastenbroek (31) observed an albino of *U. nuda* which had a low order of virulence and was believed to have arisen through mutation.

GENETICS OF THE OAT SMUTS.—In the loose and covered smuts of oats (*Ustilago avenae* (Pers.) Rostr. and *U. kolleri* Wille) certain basic characters are governed by genetic factors which bear significant relationships to species dynamics. Such characteristics as gross morphology of the smutted panicle, spore markings, pathogenicity, and culture types are known to be governed by genetic factors which frequently interact to produce wide ranges in type. Thus, type of smutted panicle may be typically loose or typically covered with a complete gradient series between. Spore wall markings may range from uniformly echinulate to smooth with intermediate types, and pathogenicity may exhibit narrow to wide host ranges with different degrees of virulence on the same host variety. Likewise, culture colonies may exhibit intermediate types between strictly mycelial and strictly sporidial. These genetic phenomena have been observed not only in experimental material but also under natural conditions.

The loose type of smutted panicle was dominant over the covered type in crosses between *Ustilago*

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avenae and *U. kolleri* reported by Hanna and Popp (14). Fischer and Holton (10) likewise observed dominance of the loose smut type in crosses between *U. avenae* and *U. perennans* Rostr.. In contrast, variability in type of smutted panicle was observed by Holton (15) in crosses between *U. avenae* and *U. kolleri*. The loose type was dominant in some crosses, the covered type dominant in other crosses, and in still others the smutted panicles were intermediate. These results support Kingsley's (26) suggestion that intermediate types encountered under natural conditions probably represent hybrids between the 2 species.

A buff mutant of *Ustilago kolleri* was reported by Holton (16), who found the smooth hyaline character of the spores to be inherited recessively in crosses with the parent species and *U. avenae* as well. In all crosses with *U. avenae* the echinulate character of the spore wall of this species was dominant over the smooth spore wall of *U. kolleri* and the buff smut. No echinulate hyaline segregates occurred in any of the crosses between *U. avenae* and the buff smut, suggesting linkage between the smooth and hyaline characters.

The heritability of pathogenicity factors in the oat smut fungi is independent of other characters and new combinations give rise to new kinds of pathogenicity. The potentialities of hybridization between *Ustilago avenae* and *U. kolleri* and their respective races are indicated by experimental results of several workers (17, 34, 41). Sampson and Western (37) and Western (46) suggested that limited opportunity for hybridization exists in nature due to the fact that infection occurs beneath the glumes from infection hyphae produced mainly by fused cells on the same promycelium. In practical experience, however, new races are encountered frequently, which suggests sufficient intermingling of distinct types to create a dynamic potentiality with respect to pathogenicity and other characteristics of the oat smuts.

GENETICS OF THE BUNT FUNGI.—The significance of genetic phenomena in relation to species dynamics in the smut fungi is perhaps best exemplified by the species of *Tilletia* occurring on wheat. In the original concept 2 species were recognized, one of which [*Tilletia caries* (DC.) Tul.] had reticulate spores and the other [*T. foetida* (Wallr.) Liro] had smooth spores. Otherwise these 2 species were virtually identical, particularly with respect to fundamental features of life cycles. Consequently, opportuntity for hybridization to occur under natural conditions is ample and there is much evidence that it does.

Genetic studies on *Tilletia caries* and *T. foetida* were initiated by Flor (11, 12) and subsequently extended by several workers (6, 18, 19, 20, 21, 22). Heritability of such characters as colony type in culture, spore wall markings, pathogenicity, rate of spore germination, size of smut ball, and number of primary sporidia was evident. The pattern of inheritance was inconsistent for all characters, the same one being dominantly inherited in some crosses and recessively inherited in others. Complementary interaction of factors produced a new kind of pathogenicity in one cross and a larger number of primary sporidia than either parent in another. The development of new pathogenic types by hybridization under field conditions undoubtedly is a major factor in perpetuating the bunt problem in the Pacific Northwest.

The nature of heritability of factors governing spore wall markings is of particular significance in relation to species variability. In some crosses the reticulate character of *Tilletia caries* was dominant while in others the smooth character of *T. foetida* was dominant. In still other crosses there were varying degrees of incomplete dominance of the reticulate character. This was true also in certain segregating populations with the result that it was possible to select a series of intermediate types differing not only from the 2 parent types but from one another as well. Similar intermediate types occur in nature and pose problems of species delimitation.

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GENETICS OF USTILAGO ZEAE IN RELATION TO BASIC PROBLEMS OF ITS PATHOGENICITY [1]

J. B. Rowell and J. E. DeVay

SUMMARY

Ustilago zeae has unique advantages for genetic investigations of the mechanisms of parasitism and pathogenicity among the smut fungi. The innumerable biotypes that arise through hybridization and mutation, the rapidity with which a single haploid cell can be increased to many billion progenies, and the short time required to complete inheritance studies have been useful in establishing important principles related to the basic problems of its pathogenicity.

Studies of mutation in cultural characters indicated that experiments with the large cell populations necessary in inheritance and pathogenicity studies must be evaluated carefully. Among black lines, abundant mutation to white and intermediate colors was found to be a barrier to precise studies of the inheritance of the pigmented character. The cell populations of mycelial lines were found by special sampling methods to contain previously unrecognized sporidial mutations. With the same methods, sporidial and pigment mutations were found to occur in presumably stable biotypes. In view of these results, real genetic purity in large cell populations must be regarded as a concept, not a reality.

Various aspects of the interrelationships among sex, parasitism, and pathogenicity in *U. zeae* have been studied with biotypes obtained by mutation and hybridization. Mutants in pathogenicity were found which exceeded the parent in virulence, and others which had lost pathogenic compatibility. The existence of multiple sex groups in this smut fungus has been confirmed through a detailed study of the inheritance of factors governing sexual compatibility. Tetropolar sex groups were found to result from the segregation of 2 pairs of factors, one with 2 and the other with multiple alleles. Amphisexual lines which possess both members of one pair of sex factors were obtained and characterized from a few crosses. Such lines were not solopathogenic. A unique pathogenic relationship was found in pairings between certain lines of *U. zeae* and *Sphacelotheca reiliana* that caused gall and chlamydospore formation in inoculated corn. No evidence of hybridization between the 2 lines was found as the chlamydospores and their monosporidial progenies through 2 "generations" resembled *U. zeae* in all characteristics. That a part of the corn smut disease syndrome was independent of dicaryon formation was indicated by the marked increase in pathogenicity of certain haploid lines that resulted when inoculated corn was given supplementary injections of the cell-free culture filtrates from a second compatible line.

Some of the outstanding problems about the nature of infectious plant diseases are those concerning the mechanisms of parasitism and of subsequent disease development. In corn smut, these problems are being investigated by genetic studies on *Ustilago zeae* in relation to the inheritance, mutability, and biochemical expression of factors controlling sex and pathogenicity.

A brief review of the life cycle illustrates the remaining basic problems pertaining to the pathogenicity of *U. zeae*. The parasitic development of the dicaryon in corn meristems is the usual pathogenic stage that results in gall and chlamydospore formation (4, 8, 20). However, solopathogenic lines which are uninucleate and probably diploid also cause galls and chlamydospores (2). Furthermore, haploid lines are also weakly parasitic under certain conditions (12), causing growth deformations but no true galls. The chlamydospores produced in the galls contain a single diploid nucleus, and meiosis occurs during germination and promycelium formation (8). There are many variations in forms of promycelia (7), but the typical form is 4-celled, each cell bearing a lateral sporidium. Monosporidial isolation of the sporidia from promycelia demonstrates segregation of factors for sexual (2, 4), cultural (22), morphological (6), pathological (4, 22) and biochemical (13) characteristics. The sporidia grow readily saprophytically as auxo-autotrophs (19), i.e., requiring only an organic carbon energy source, and the characteristics of such cultures have been studied extensively. That multiple sex groups occur among monosporidial isolates has been demonstrated by Stakman et al (22), Hanna (4), Christensen (2) and others. Fusions between sporidia of compatible lines in vitro have been reported (1, 20), but they are not readily observed. The dicaryon resulting from fusions between sexually compatible lines has not been successfully perpetuated in culture and obviously differs in its physiological requirements from the haploid cultures.

There are, therefore, a number of unanswered questions on the pathogenicity of *U. zeae*. Why does fu-

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sion of the haploid sporidia cause a change from a saprophytic to a parasitic habit? Are the processes of sex and pathogenicity governed by the same or separate genic factors? What are the pathogenic mechanisms causing gall formation? Recent progress made in the genetics of these problems at Minnesota will be reviewed herein.

ROLE OF MUTATION IN STUDIES OF INHERITANCE AND PATHOGENICITY.—Stakman and coworkers have demonstrated that *U. zeae* comprises an indefinite number of haploid biotypes and that new ones continually arise by mutation and hybridization (22, 23, 24, 25). Approximately 15,000 biotypes have been isolated and studied in the Minnesota laboratory during the past 30 years. To study the inheritance and pathogenicity of biotypes it is necessary to inoculate corn seedlings with actively growing cell mixtures of 2 compatible haploid lines. Therefore, a basic problem in such investigations is to determine how mutation occurring in the population after or during the process of inoculation and growth in the corn plant affects the processes of hybridization and segregation.

Recently, Lu (10) undertook a quantitative study of the inheritance of factors for color in crosses between black lines and white ones. Frequent sectoring occurred in all black lines studied and indicated that they were extremely mutable. By special methods of dispersing individual cells or mycelial fragments on the surface of solid agar, many distinctly different color types were found to be produced in a single culture (Fig. 1). When cultures were started from single cells it was found that the rate of mutation from black to white lines in 3 monosporidial black lines was approximately 0.1 per cent. The white mutants multiplied at the same rate as the black parent and also mutated frequently to other pigmented types. Consequently, a very heterogeneous population of mutants with white and intermediate colors was produced during the growth from a single cell to 10 billion sporidia in a single cultural generation. It is evident that crosses would mate mixed populations of biotypes. Crosses were made between black and white lines, and the segregates of the resulting chlamydospores were sampled by the isolation of individual sporidia from promycelia and by the special plating method. White, intermediate, and black pigmented types were recovered from crosses of black × black, black × white, and white × white in various ratios. Since the pigment was produced by tyrosinase activity, theoretical interpretations of the inheritance of color could be made of the factors for color base (phenolic compounds), color activators (tyrosinase), and color inhibitors (ascorbic acid). Actually, the different color types could be produced by mutation after or during the inoculation of the corn plant; therefore, it was impossible to determine the relative importance of recombination and mutation in the resulting lines. The results confirmed the earlier conclusions of Stakman and co-workers that abundant mutation constitutes a formidable barrier to precise studies on the inheritance of color (22, 23).

It had recently been shown that mutants may be present in monosporidial lines without any visible evidence of their presence until special methods were used for their detection. Although mycelial mutants were produced frequently during the growth of sporidial biotypes on agar substrates, mutations of mycelial to sporidial growth types rarely were observed (22). In certain mycelial lines, Miss Jackson and Rowell (5) found that mutation to sporidial-growth types occurs within approximately 10^5–10^8 divisions in broth cultures, whereas the mutants were not observed in the agar cultures. Studies of competitive growth in mixtures established that the mycelial parent had a growth advantage on agar media and the sporidial mutant in the broth cultures. Therefore, the presence of the mutants was obscured by the environmental conditions and special methods were required for their detection.

Even in stable lines it was found that mutation resulted in a mixed population of a number of biotypes. Among the corn smut cultures in the Minnesota collection is 10A4, which was considered to be a pure biotype. In continuous laboratory culture since 1934, the distinctive characters of mycelial growth type, felt-like surface, and pale vinaceous pigmentation of 10A4 had remained constant, although mutations were induced with various mutagenic treatments by Petty (14) and others. Recently the occurrence of natural sporidial (Fig. 2) and color mutations were demonstrated in line 10A4 by the use of the special plating technique. Apparently, cultures of stable lines of *U. zeae* cannot be maintained as pure biotypes.

Results of studies of mutation in cultures of *U. zeae* have established principles that are important in interpreting the behavior of monosporidial lines. The rapidity with which very numerous progenies are produced and the frequent mutation of many characters resulted in mixed populations even in what were apparently stable biotypes. The presence of some mutations may be unsuspected without special methods for their detection. In view of these facts, inheritance studies of sex and pathogenicity with *U. zeae* must be evaluated carefully for artifacts due to mutation.

GENETICS OF PATHOGENICITY AND SEX.—The experience of Stakman, Tyler, and Hafstad (25) that mutants of *U. zeae* can exceed the parent in virulence and that others lose pathogenic compatibility were substantiated. In recent tests of 932 natural and induced mutants of haploid lines, many mutations in degree of virulence were found (Fig. 3), most of which were less pathogenic than the parent. However, some mutants were materially greater than the parent in pathogenicity. Changes in the relative virulence of a line could have resulted from mutations either in the actual characters of pathogenicity or in any of the characters affecting physiologic vigor. One mutant was found which failed to produce galls in crosses with any line of the many sex groups tested. Here, either sexual or pathogenic characters might have changed. As the

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TABLE 1.—*Compatibility of sex groups isolated from a typical chlamydospore collection*

Sex Groups	Cross reaction in inoculated corn				Factors
	I	II	III	IV	
I	—[a]	—	—	+	a^1b^1
II	—	—	+	—	a^1b^2
III	—	+	—	—	a^2b^1
IV	+	—	—	—	a^2b^2

[a] — indicates negative results; + indicates gall and chlamydospore formation.

TABLE 2.—*Compatibility of sex groups from several chlamydospore collections*

Collection Source[a]	Factors	Cross reaction in inoculated corn					
		NyBb a^2b^2	NyBg a^2b^4	InC2 a^2b^3	InC4 a^2b^5	BrA4 a^2b^7	BrA3 a^2b^6
NyAd	a^1b^3	—[b]	+	+	+	+	+
NyCe	a^1b^4	+	—	+	+	+	+
InB4	a^1b^8	+	+	—	+	+	+
InC4	a^1b^5	+	+	+	—	+	+
BrA1	a^1b^7	+	+	+	+	—	+
BrA2	a^1b^6	+	+	+	+	+	—

[a] Collection source indicated by first 2 letters: Ny = New York; In = Indiana; Br = Brazil.

[b] — indicates negative results; + indicates gall and chlamydospore formation.

parasitic dicaryon results from fusion between cells of 2 sexually compatible lines, a knowledge of the genetic factors controlling sex is necessary to an understanding of its role in pathogenicity studies.

Heterothallism in *U. zeae* was discovered by Stakman and Christensen (21) and the existence in this species of multiple sex groups was established by Stakman et al (22), Hanna (4) and Christensen (2). The nature of sex in this smut was recently reviewed by Whitehouse (26) who concluded that *U. zeae* is bisexual. However recent studies with monosporidial lines from 11 wild chlamydospore collections have confirmed the earlier reports of multiple sex groups in *U. zeae*. The production of fertile zygotes (chlamydospores) which occurs only in infected corn was considered the ultimate criterion of sexual compatibility. The partial vacuum method of inoculating germinating corn seedlings (17) was used to make crosses. All possible combinations among the monosporidial isolates from the chlamydospores of a single wild sorus or domestic cross separated the lines into at least 4 sex groups among which there were only 2 compatible combinations (Table 1). Hanna (4) represented this behavior on a factorial basis as the segregation and recombination of 2 pairs of factors. In the present study, the factors were designated as a^1a^2 and b^1b^2, and compatible combinations mated different alleles at the "a" and "b" loci. Thus only $a^1b^1 \times a^2b^2$ (Groups I × IV) and $a^1b^2 \times a^2b^1$ (Groups II × III) produced galls and chlamydospores. When lines representative of the 4 groups from 11 different chlamydospore sources were mated in all possible combinations only 2 forms of the "a" factor were found. Thus, there were 2 large groups of lines within which there were no compatible matings, i.e. lines having factor a^1 or factor a^2 in common. Matings between these 2 groups, however, divided them into many smaller groups in which those mating similar b's were incompatible, but all remaining combinations produce galls and chlamydospores (Table 2). There were 15 different "b" factors among these lines. Monosporidial isolates from the chlamydospores of representative crosses were mated with a line from each sex group and in all possible combinations with each other. From most crosses only the 4 recombinations of the "a" and "b" factors were found. To date, no lines were found in which 2 different "b" factors were combined with one of the "a" factors in 19,719 tests of compatibility with 1305 lines from 62 crosses. Therefore, the "b" locus was concluded to have multiple alleles.

The situation in regard to sex proved to be still more complicated when some unusual mating types were isolated from occasional crosses. Thus 7 groups were found in the monosporidial isolations from chlamydospores of the cross of 10A4 × 17D4 and the solopathogen 410qq, isolated from this cross (Table 3). Four groups corresponded to the haploid recombinations of the 2 pairs of compatibility factors previously described. A fifth group of lines was solopathogenic and like 410qq had the diploid complement of sex factors $a^1a^2b^1b^2$. The remaining 2 groups were called amphisexual as they crossed with all a^1 and a^2 haploid lines except those possessing either b^1 for 1 group and b^2 for the other. The presence of both the a^1 and a^2 factors in these lines was established by hybridization studies. Furthermore, when amphisexual lines were exposed to alpha radiation, variants were obtained which had the mating reaction of typical a^1 and a^2 haploid lines (18). The combination of the 2

FIG. 1–4. Fig. 1. "Surface-dispersed" plate of a sample of the population of a monosporidial black line illustrating the occurrence of several white mutants (Lu (10)). Fig. 2. Comparison of "stable biotype" 10A4 (A) and the natural sporidial mutant (B) found in cell populations. Fig. 3. Comparison of the pathogenicity to corn of line 17D4 and 3 mutants in crosses with 10A4; (A) 17D4-19-20 (A) without pathogenic compatibility, (B) parent line 17D4, (C) 17D4-8-13 with virulence comparable to parent, and (D) 17D4-10 with virulence exceeding parent. Fig. 4. Stimulation of curl reaction in combinations of sporidia of one haploid line + culture filtrate of a second compatible haploid line, (A) 10A4 cells + 17D4 filtrate, (B) 10A4 cells, (C) 10A4 cells + 17D4 cells, (D) 17D4 cells, (E) 17D4 cells + 10A4 filtrate (DeVay (3)).

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TABLE 3. *Unusual sex groups isolated from chlamydospores of 10A4 × 17D4 and its solopathogen 410qq*

No. of isolates		Cross reaction with tester lines in inoculated corn				Solo-	
10A4 × 17D4	410qq	a^1b^1	a^1b^4	a^2b^4	a^2b^1	pathogenic	Factors
1	6	+[a]	—	—	—	—	a^2b^4
2	8	—	+	—	—	—	a^2b^1
9	6	—	—	+	—	—	a^1b^1
1	10	—	—	—	+	—	a^1b^4
2	1	+	—	—	+	—	$a^1a^2b^4$
12	1	—	+	+	—	—	$a^1a^2b^1$
26	1	+	+	+	+	+	$a^1a^2b^1b^4$

[a] + indicates gall and chlamydospore formation; — indicates negative results.

"a" factors in a single non-solopathogenic line clearly indicated that they were not primary pathogenic characters. The existence of partial diploids for the "a" sex factors indicated the possibility of obtaining such combinations of other characteristics for sex and pathogenicity. An unsuccessful search was made for similar combinations of the 2 different "b" factors in a single line to determine their relationship to pathogenicity.

SEPARATION OF FACTORS FOR SEX AND PATHOGENICITY.—Certain characteristics of pathogenicity can be separated from the factors for sex. Stakman and coworkers (23, 24) in studies of the inheritance of a white character in 39 distinct monosporidial lines found that white × white crosses produced the dicaryophase and large galls, but not the diplophase and chlamydospores. The white lines were demonstrated to be of different sex groups by the production of chlamydospores in crosses of these lines with pigmented tester lines. They concluded that the crosses of white lines possessed factors for the formation of the dicaryophase and pathogenicity, but lacked the necessary factors for nuclear fusion and the production of zygotes.

Rowell (16) has reported the pathogenicity for corn of certain pairings of haploid lines of *U. zeae* with *Sphacelotheca reiliana*. Partial vacuum inoculation of the meristems of growing corn seedlings with these pairings resulted in the production of distinct galls which were smaller and slower in rate of development than those produced by a normal cross of compatible *U. zeae* lines. By isolations from the galls, both smut lines were recovered, and their joint pathogenicity demonstrated by reinoculation. Although chlamydospores were rarely produced in these galls, they were obtained from 8 different pairings. The size, echinulations and germination of the spores were all similar to those of the *U. zeae* parent. Furthermore, all monosporidial isolates obtained from the spores were identical to the *U. zeae* line in cell morphology, cultural characters, sex factors, and in reaction with the *S. reiliana* lines (Table 4). None of the isolates produced galls when paired with one another or with the original *U. zeae* line. Back pairing of these isolates to the *S. reiliana* line again produced galls, chlamydospores, and monosporidial isolates similar in all respects to those of the first pairing. Thus, there was no evidence of segregation found for any characters that were studied. Galls in various stages of development produced by the *U. zeae* and *S. reiliana* pairings were sectioned, stained, and examined, but only uninucleate mycelium was found. Although the potentiality for pathogenic compatibility between the 2 smut fungi was associated with certain sex factors, no evidence of hybridization was found. The results suggested that inoculations with these paired lines resulted in a physiologic synergism that incited gall formation without fusions to form the dicaryophase or diplophase and the gall physiology stimulated the *U. zeae* line to form chlamydospores. However, an abnormal genetic relationship may exist between the lines.

The weak parasitism of single haploid lines was another means of studying pathogenic characteristics independently of sex. Certain haploid lines were found by Munnecke (12) to cause curl reactions that were distortions of corn tissue similar to the hyperplastic phase of normal infection. Infection of the tissues was demonstrated cytologically and by reisolation of the original lines. Furthermore, combinations of 2 lines which individually had weak reactions resulted in more severe symptoms, yet no true galls or chlamydospores were formed. Recently, DeVay (3) attempted to supplement the "missing portion" of the gall stimulus by inoculating corn seedlings with cells of a single haploid line plus the cell-free filtrate from a second compatible haploid line. Although some galls with chlamydospores were obtained, further experiments to duplicate the results were unsuccessful. However, the degree of distortion produced by a haploid line alone consistently was increased by adding the cell-free filtrate from a second compatible line (Fig. 4). Supplements of sterile media were ineffective. The growth responses obtained by these treatments were undoubtedly a part of the disease syndrome and growth regulators were suspected. The synthesis of indole acetic acid and possibly other auxins by *U. zeae* in artificial culture has been reported by Link et al (9), Moulton (11), and Wolf (27). However, no host reaction was obtained from the various combinations of cell-free culture filtrates tested.

If growth regulators had a primary role in the formation of corn smut galls, it should be apparent from stimulation or inhibition of disease development by

TABLE 4. *Pathogenic and sexual compatibility among Ustilago zeae, Sphacelotheca reiliana and derived lines*

	Reaction of pairings in inoculated corn							
	U. zeae				*S. reiliana*			
U. zeae	NyCe	NyAd	17D4	OhAf	SrGa	SrLb	SrJa	SrMb
NyCe (a^1b^4)	—[a]	—	—	+	—	—	—	—
NyAd (a^1b^2)	—	—	+	—	—	—	—	—
17D4 (a^2b^4)	—	+	—	—	—	—	—	—
OhAf (a^2b^2)	+	—	—	—	+	+	—	—
S. reiliana								
SrGa	—	—	—	+	—	—	S	—
SrLb	—	—	—	+	—	—	S	—
SrJa	—	—	—	—	S	S	—	—
SrMb	—	—	—	—	—	—	—	—
Four serial isolates from a chlamydospore of *SrGa* × *OhAf*								
1 A1	+	—	—	—	+	+	—	—
A2	+	—	—	—	+	+	—	—
A3	+	—	—	—	+	+	—	—
A4	+	—	—	—	+	+	—	—

[a] — indicates negative results; + indicates gall and chlamydospore formation, and S indicates sori formation in tassels and ears.

supplements of auxins or auxin inhibitors. Rowell (unpublished) attempted to alter gall formation in plants inoculated with a virulent and a weak pathogenic entity. In the partial vacuum method of inoculating seedling corn, the cross of haploid lines 10A4 × 17D4 consistently produced large stem galls that involved more than 60 per cent of the aerial plant parts. The solopathogen 410n, however, produced mostly small leaf or stem galls that occupied 20 per cent of the aerial plant parts (17). Attempts to increase the proportion of gall tissue in the 410n inoculated plants with various growth regulators were negative. Conversely, auxin inhibitors did not suppress markedly the proportion of gall formation in plants inoculated with 10A4 × 17D4. Various dosages of the test substances (examples: indole acetic acid, alpha naphthalene acetic acid, Na 2, 4 dichlorophenoxyacetate, 2, 3, 4, triiodobenzoic acid, trans cinnamic acid, coumarin, dichloroanisole) were applied to the plants as foliage sprays, soil drenches, vacuum impregnation, and injection to the seedling meristems in lanolin-corn oil mixtures with essentially the same results. That growth regulators could cause proliferation in corn was indicated by gall-like growths induced in the area of the adventitious root initials at the coleoptile node of uninoculated plants treated with 1:10,000 Na 2,4 dichlorophenoxyacetate as a soil drench. However, experiments with inoculated plants indicated that these growth regulators did not control gall formation in corn by *U. zeae*.

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INDUCTION AND INHERITANCE OF MUTANT CHARACTERS IN VENTURIA INAEQUALIS IN RELATION TO ITS PATHOGENICITY [1]

G. W. Keitt and D. M. Boone

SUMMARY

Numerous morphological, color, and biochemical mutants of *Venturia inaequalis* (Cke.) Wint. were induced by treatments with the nitrogen mustard methyl-bis (Beta-chloroethyl) amine or ultraviolet irradiation. Many of the mutant characters were studied genetically. Segregation was usually in 1:1 ratio. Numerous mutant genes were located with reference to their respective centromeres, and linkage groups were established. Many biochemical mutants were characterized for their respective deficiencies, which fell into the categories of vitamins, nitrogen bases, amino acids, and reduced sulfur. The biochemical mutations had effects on pathogenicity that ranged from no apparent change in some cases to complete suppression in others. Further studies are in progress.

The needs for research on the genetics of pathogenic organisms in relation to basic problems of infectious disease have been discussed by Stakman (43), Keitt and Langford (21), and Keitt (16), who have pointed out that phytopathogenic fungi offer exceptionally favorable material for such work.

Substantial progress has been made in genetic studies with phytopathogenic fungi, and much of the earlier work in this field has been reviewed (9, 10, 13, 33, 44). Chief attention was first given to the rust and the smut pathogens (10, 13, 33), which have many excellent adaptations for this work; however, other fungi offer some important advantages of a different kind. The investigations of Dodge and coworkers (11, 12, 41 and papers cited in 28), Lindegren and colleagues (27, 28), Beadle and associates (2, 3), and others (see 8) greatly advanced knowledge of certain nonpathogenic Ascomycetes in the genus *Neurospora* and drew attention to some rare adaptations of this type of Ascomycete for genetic and biochemical studies.

Work of the present writers and associates and of others has shown that the apple scab fungus, *Venturia inaequalis* (Cke.) Wint., an Ascomycete with many similarities to the 8-spored Neurosporas, has excellent adaptations for correlated genetic, physiologic, and biochemical studies in relation to basic problems of infectious disease. These researches in our laboratory were primarily concerned at first with laying a groundwork for genetic studies with this pathogen and next

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The papers of the writers and coauthors have been freely drawn upon. Grateful acknowledgments are made to the many coworkers who have participated in these investigations. These studies were supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation and also in part by a grant E-140 (C) from the National Microbiological Institute of the National Institutes of Health, Public Health Service.

with the inheritance of its pathogenicity. In the past several years our program has been broadened to include correlated studies on the genetics, nutrition, and pathogenicity of this fungus. The present paper contains a report of progress of certain phases of these later studies. Brief reports on some of the work dealt with have been published (5, 6, 7, 14, 17, 18, 19), and a fuller account of it will be given in other papers.

Since a review of literature relating to genetics of *V. inaequalis* has recently been published (15), very brief reference to the earlier work will furnish sufficient background for the present report.

In 1916 one of us began researches on apple scab that have been continued throughout the intervening years. Studies of the epidemiology and control of the disease led to investigations of the inciting fungus and of techniques and phenomena of infection (20). In these and later investigations (30, 31) in our laboratory, and independently by Wiesmann (45), it was shown that different isolates of the fungus have different pathogenic capabilities. These findings were confirmed and extended by Rudloff (34), Schmidt (35, 36, 37, 38, 39) and Küthe (25). Nusbaum and Keitt (29) made a cytological study of host-parasite relations involving susceptible, intermediate, and resistant host reactions. Keitt and Palmiter (24) showed that the pathogen, an 8-spored Ascomycete, has a true sexual stage with 2 mating types, and can be cross-bred experimentally. Backus and Keitt (1) made a cytological study of some nuclear phenomena in *V. inaequalis*, showing that the vegetative cells are uninucleate, that the 4 spores at either end of the ascus ordinarily derive their nuclear complements from 1 first-division nucleus, and that the sister nuclei or spores ordinarily lie next to each other in the ascus, thus making it possible to trace the line of nuclear descent after the methods worked out by others with *Neurospora*. Keitt and Langford (21) developed techniques for breeding the pathogen *in vitro* and opened up genetic studies, with special reference to inheritance of pathogenicity and of mating type. The constancy of genetic behavior of the fungus was critically examined and shown to compare well with that of higher plants and animals. It was shown that this fungus has excellent adaptations for genetic and physiologic studies similar to those of the 8-spored Neurosporas but including the aspect of pathogenicity, which *Neurospora* lacks. They found that factors for control of pathogenicity segregated in 1:1 ratio alternatively in the first or the second nuclear division in the ascus. Keitt, Langford, and Shay (22) reported that only 2 major types of pathogenic reaction, designated as "lesion" (typically pathogenic, with abundant sporulation of the fungus) and "fleck" (non- or only slightly pathogenic, with sporulation lacking or very sparse), were encountered with wild-type lines (Fig. 1). In all cases adequately studied, segregation of factors for pathogenicity as determined by the lesion and fleck reactions was in 1:1 ratio, alternatively in the first or the second nuclear division in the ascus. Studies of progenies from crosses of the cultural mutant *tan* and the double mutant *tan non-conidial*, respectively with *normal* showed that these mutant genes suppressed or modified the expression of pathogenicity. Recovery of the pathogenicity of the line from which these mutants arose showed that the mutations concerned did not change the gene conditioning pathogenicity. Shay and Keitt (40) studied inheritance of mating type and of certain mutant characters and began chromosome mapping. Keitt, Leben, and Shay (23) made further studies on inheritance of pathogenicity, employing 4 apple varieties and 2 compatible lines of the fungus with different pathogenic capabilities for these varieties. In each ascus tested, segregation for pathogenicity to each variety was in 1:1 ratio; analysis indicated that pathogenicity to 2 varieties was controlled at 1 locus and to the other 2 at another locus. There was no evidence of linkage of these loci. Schmidt (39) has reported experiments from which he concluded that inheritance of virulence in *V. inaequalis* to given apple varieties is complex. However, his pathogenicity tests were conducted out of doors with limited opportunity to control environment. Furthermore, he did not exclude possible effects of modifying factors. Leben and Keitt (26) studied the influence of carbon and nitrogen sources and of vitamins on growth of *V. inaequalis in vitro*. A wide range of carbon or nitrogen sources could be utilized with different lines of the fungus showing different degrees of efficiency in utilization. The fungus was partly deficient for only 1 of 17 vitamins studied, thiamine. When thiamine was present, "vitamin-free" casein hydrolysate increased growth of all lines of the fungus tested. No differences in carbon, nitrogen, or vitamin nutrition of the fungus were clearly associated with differential pathogenic capabilities of the lines studied. Keitt and Boone (18, 19) have reported additional gene pairs conditioning pathogenicity in wild-type lines of

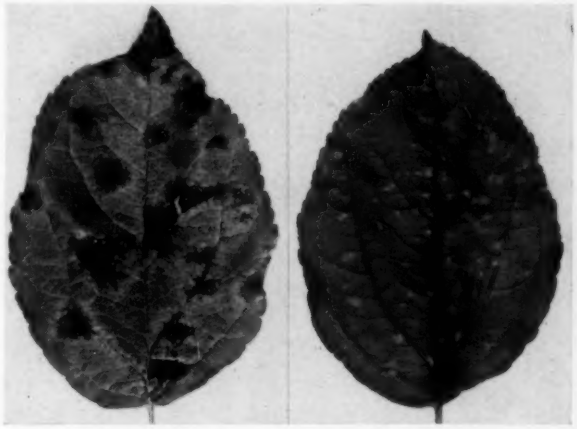

FIG. 1. Apple leaves inoculated with *V. inaequalis*, showing characteristic lesion (left) and fleck (right) reactions.

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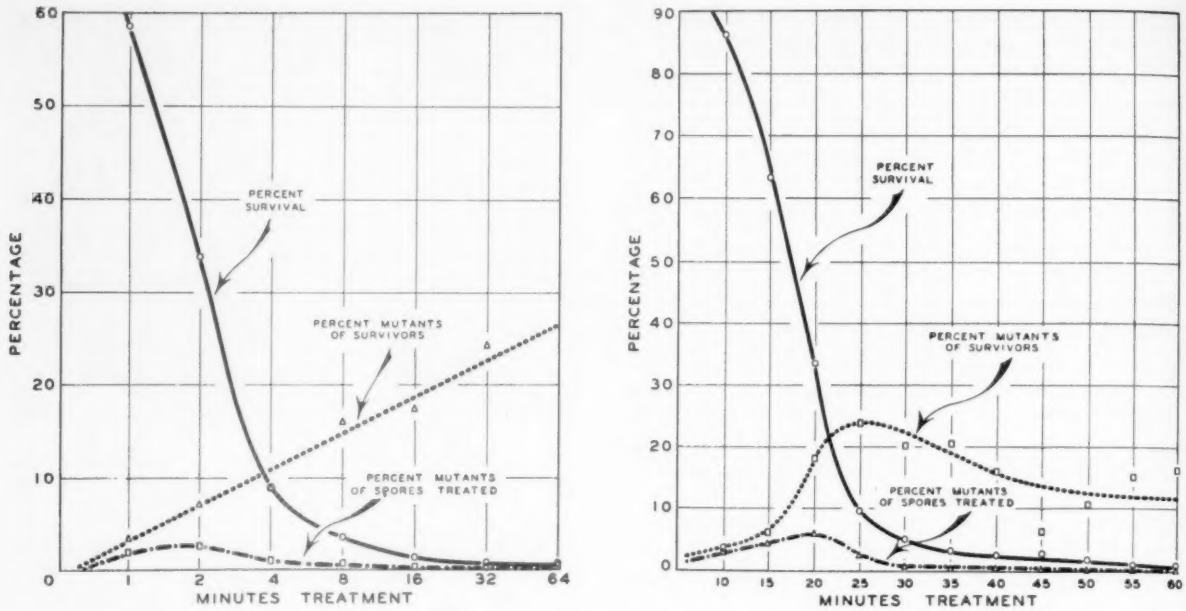
PERCENTAGE
PERCENT SURVIVAL
PERCENT MUTANTS OF SURVIVORS
PERCENT MUTANTS OF SPORES TREATED
MINUTES TREATMENT
0 1 2 4 8 16 32 64
10 20 30 40 50 60
0 10 20 30 40 50 60 70 80 90
10 15 20 25 30 35 40 45 50 55 60

FIG. 2. Summary of results from treatments of conidia with nitrogen mustard (left) and ultraviolet light (right).

the fungus. Pelletier and Keitt (32) studied growth responses of the fungus to 19 amino acids, which they grouped according to efficiency as nitrogen sources. They suggested that amino acid nutrition may be important in relation to pathogenicity.

The foregoing work has shown the following special advantages of *V. inaequalis* for the investigations herein reported: A) It freely infects young, vigorous, unwounded tissues and may live for many weeks in intimate association with living host cells. B) It has many bio-types with different pathogenic capabilities to different host species or varieties. C) It can be cultured throughout its life cycle and cross-bred at will *in vitro*. D) It can be maintained for many years *in vitro* with satisfactory stability of the characters studied. E) It is haploid throughout the parasitic phase and in its vegetative stage *in vitro*, thus permitting study of effects of a single set of genes. F) The ascus contains the complete progeny stemming from a single meiosis, with survival of all nuclei in an orderly arrangement that permits tracing the line of nuclear descent by isolating the ascospores in their serial order and studying the derived cultures. G) The vegetative cells are uninucleate and free from important problems of heterocaryosis. H) Materials and methods have been developed for correlated studies on its genetics, nutrition, and pathogenicity.

Our earlier work with mutants was concerned with those that arose spontaneously in cultures *in vitro*. The outstanding accomplishments in inducing mutations of *Neurospora* and studying their effects on biochemical processes (2, 3, 4) suggested that similar studies with *Venturia* might give new approaches to the nature of its parasitism and related problems.

Mutations in the apple scab pathogen were induced[2] consistently by treatments with either the nitrogen mustard methyl-bis (beta-chloroethyl) amine (42) or ultraviolet irradiation. A fuller account of the methods employed and the results obtained will be given elsewhere. The results of 2 such treatments are shown in Fig. 2. In the nitrogen mustard treatments the percentage of mutants among survivors tended to increase throughout the treatment period; in

[2] Grateful acknowledgments are made to M. H. Stahmann for giving the nitrogen mustard treatments and to J. F. Stauffer for performing the ultraviolet irradiation.

TABLE 1.—*Biochemical mutants obtained from mutagenic treatments of V. inaequalis*

Deficiency	Number of mutants	Probable number of loci represented[a]
Vitamins		
Nicotinic acid	1	1
Biotin	1	1
Inositol	3	1
Pantothenic acid	3	1
Choline	5	1
Riboflavin	6	2
Nitrogen bases		
Pyrimidines	19	4
Purines	17	8
Amino Acids		
Arginine	18	4
Lysine	1	1
Histidine	3	2
Methionine	4	—
Proline	2	—
Reduced sulfur	16	3

[a] See text for methods of determining the number of loci.

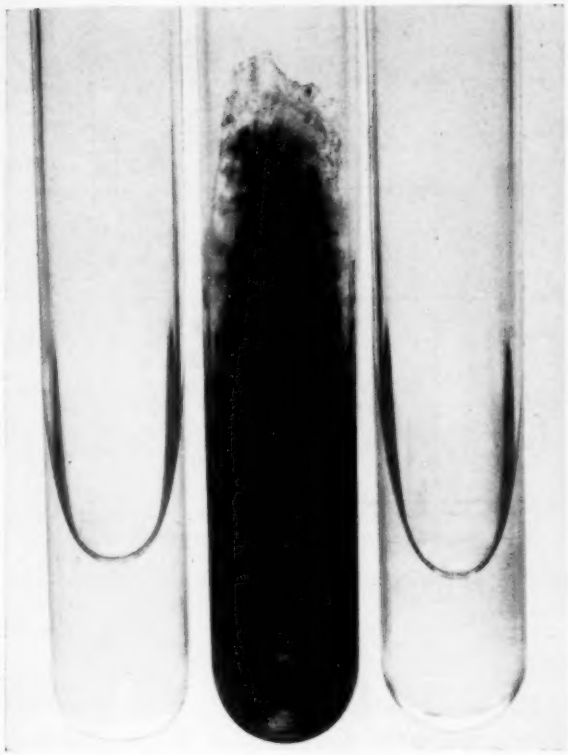

FIG. 3. Growth of 2 biochemical mutants cultured together (center) and separately (left and right) in liquid minimal medium.

the ultraviolet irradiation treatments the percentage of mutants among survivors tended to reach a maximum and then decline. The ultraviolet treatments, which are more convenient, have been employed in most of the later work.

The mutations induced were of 3 general types: morphological, color, and biochemical. The morphological mutants were the most numerous but were the least useful for the purposes of our investigations. Many of the morphological mutations affected the rate and extent of growth; some affected the character of the mycelium, the amount of sporulation, or the size and shape of the conidia. Genetic studies were made with certain of these mutant characters, such as *dendritic*, *dwarf*, *nonconidial*, *round spore*, and *small*.

Striking color mutants were obtained whose color characters served as excellent markers in genetic studies. These mutants were brown, green, pale, pink, white, or yellow. Brief reports on the inheritance of representative color mutant characters have been made (5, 6), and a fuller account of this work will be published elsewhere. The color mutant characters were used to establish 3 linkage groups as follows: group 1 with the loci of *white*-2, *pale*, *brown*, and the centromere; group 2 with the loci of *pink*, *yellow*-2, *green*, and the centromere; group 3 with the locus of *yellow*-1 and the centromere. Other loci have been

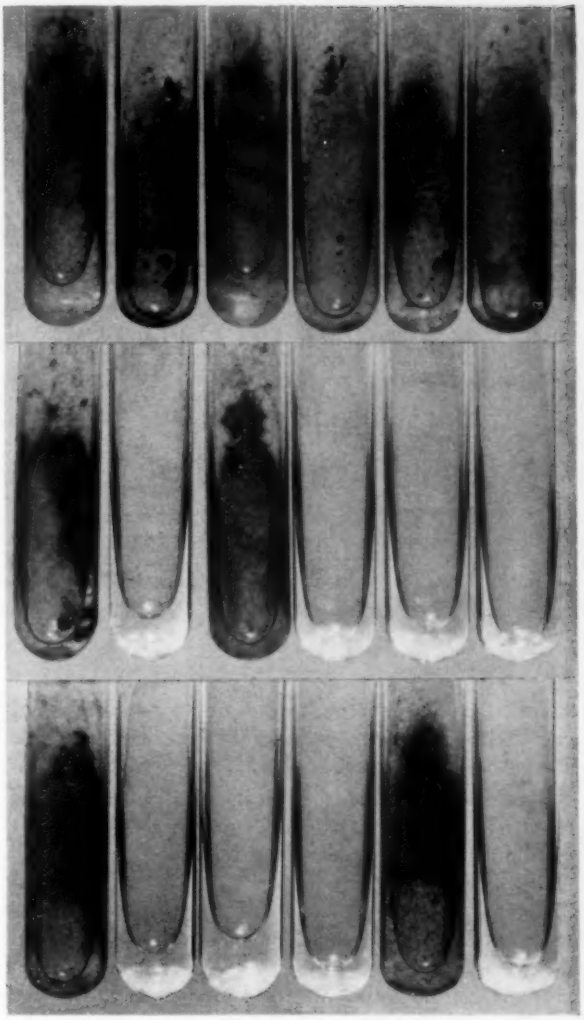

FIG. 4. Growth of wild-type (top) and 2 biochemical mutants (middle and bottom) in liquid media used in classifying amino acid deficiencies. The media from left to right are: minimal plus casein hydrolysate, minimal alone, and minimal supplemented, respectively, with 4 groups of amino acids.

added to these groups through studies with morphological and biochemical mutants.

The biochemical mutants were of special interest in relation to pathogenicity. A brief report on them has been made (7). A list of the biochemical mutants induced in *V. inaequalis* is in Table 1. The probable number of loci of mutant genes affecting the requirement for the same chemical was determined by studies of segregation of the mutant characters in crosses and by tests of the ability of pairings of mutants to grow synergistically in a liquid minimal medium consisting of inorganic salts and dextrose in distilled water. The results of 1 of these tests for synergy are illustrated in Fig. 3. Good growth of the pairs of mutants in minimal medium was regarded as evidence that the

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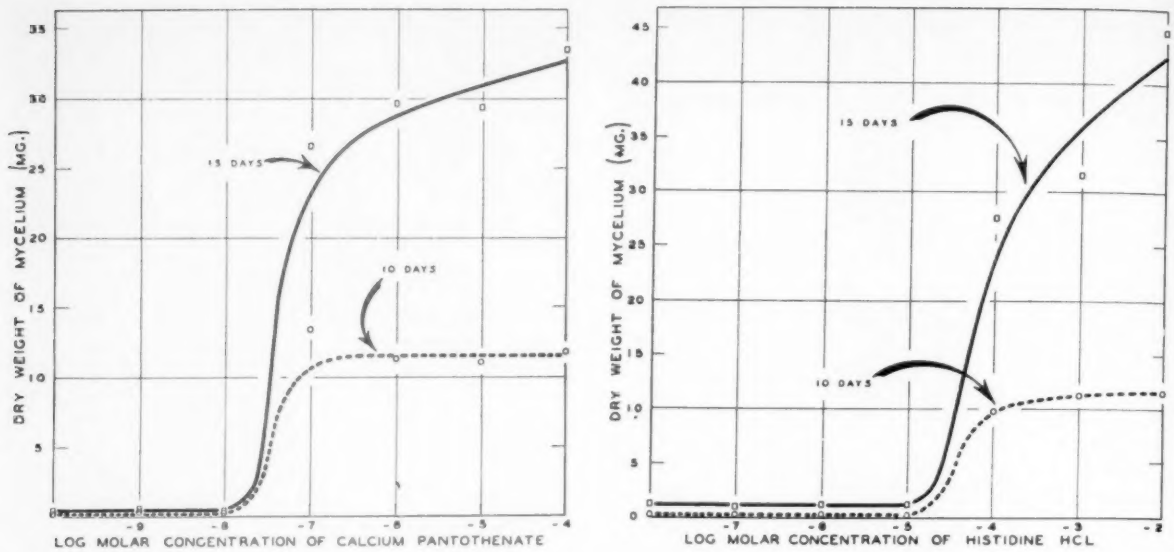

FIG. 5. Growth of 2 biochemical mutants deficient, respectively, for pantothenic acid (left) and histidine (right) with different concentrations of the required chemical.

mutant genes concerned were not identical. Final conclusions as to the number of loci will be made after studies of recombination.

The deficiencies in the biochemical mutants here reported appeared to be complete. No growth or only a slight trace of growth was observed for any of these mutants when attempts were made to grow each individually in liquid media lacking the chemical for which there was the deficiency. Fig. 4 illustrates the growth of a wild-type line and 2 biochemical mutants in minimal medium alone and minimal medium supplemented with casein hydrolysate or with groupings of amino acids. Quantitative measurements of growth of some of the mutants have been made by determining the dry weight of the mycelium produced with different concentrations of the required chemical. Growth response curves for 2 of the mutants are shown in Fig. 5. This definitive response of the mutants to the presence or absence of the required chemicals, coupled with their inherent pathogenic capabilities, makes feasible their use in *in vivo* tests for determining the availability of these substances in the living host tissue.

The inheritance of 54 of the biochemical mutant characters has been studied. Each of the deficiencies appeared to be single-gene controlled, but there was evidence that a few of the biochemical mutants also carried morphological mutant genes. Table 2 lists some of the mutants studied genetically and shows the distance of the loci from their respective centromeres as calculated from the data thus far obtained on the percentage of second-division segregation of the mutant characters. A fuller treatment of these and additional data will be given in a later paper.

Tests were made for linkage between biochemical mutant genes and those of the 3 linkage groups mentioned previously. The *nicotinic acid* gene in group 1 was the only one found to be closely linked with any of the color mutant genes. Tests of recombination between biochemical mutant genes are in progress. Since cytological studies (1) have indicated that the probable haploid chromosome number in *V. inaequalis* is of the order of 6, there is strong possibility that from among the many color, morphological, and biochemical genes at hand, genetic markers may be available for each of the chromosomes.

By means of techniques and equipment previously described (21) inoculation experiments were made to study possible effects of the mutations on the pathogenicity of the fungus. Special interest attaches to such effects in the case of biochemical mutants, because of the opportunity afforded to study known specific nutritional deficiencies of the pathogen in relation to the disease reaction with its host.

Comparatively little attention has been given to the pathogenicity of the morphological mutants, since they seemed to offer less favorable material for our purposes than other available mutants.

In general, the color mutants incited the same pathogenic reactions as did the wild-type lines from which they were derived. However, the sporulating lesions had the characteristic colors of the mutants that incited them. Two mutants, *white*-1 and *yellow*-2, showed reduction of pathogenicity similar to that of the cultural mutant *tan* (*v. s.*). As in the case of *tan*, studies of progeny from crosses of each mutant with *normal* showed that the mutations suppressed expression of the genes conditioning the pathogenic reaction but did not change them.

TABLE 2.—*Summary of data on the distance from the centromere of representative biochemical mutant genes*

Deficiency	Mutant No.	Number of asci studied	Second-division segregation (per cent)	Distance from centromere in cross-over units[a]
Vitamins				
Biotin	2570	47	78.7	——
Choline	1905	54	87.0	——
Inositol	2694	46	10.9	5.5
Nicotinic acid	2599	79	16.5	8.3
Pantothenic acid	2584	56	91.1	——
Riboflavin	2343	61	14.8	7.4
Riboflavin	3115	48	47.9	24.0
Nitrogen bases				
Pyrimidines	953	22	54.5	——
Pyrimidines	1907	182	15.9	8.0
Pyrimidines	2481	85	10.6	5.3
Pyrimidines	2340	72	47.2	23.6
Purines	950	36	69.4	——
Purines	2106	90	55.6	27.8
Purines	2370	24	58.3	——
Purines	2702	42	81.0	——
Purines	2892	54	22.2	11.1
Purines	3006	26	84.6	——
Purines	3010	59	11.9	6.0
Amino acids				
Arginine	1119	57	1.8	.9
Arginine	2996	54	51.9	26.0
Arginine	3072	29	72.4	——
Histidine	1261	76	81.6	——
Histidine	1336	90	6.7	3.4
Methionine	2602	32	75.0	——
Proline	2366	40	62.5	——
Reduced sulfur	1901	78	28.2	14.1
	2302	50	76.0	——

[a] Distances, uncorrected for double cross-overs, are given only for those characters whose percentage of second-division segregation was significantly less than 66.7. See text.

The results thus far available from inoculation studies to test the pathogenicity of the biochemical mutants are shown in Table 3. It should be noted that all the biochemical mutants reported in this paper were derived from 1 monoascosporic line of the fungus that incited typical lesions on leaves of the apple varieties used in the inoculation experiments reported in Table 3. If the typical lesion reaction is obtained in these tests, it is clear that under the experimental conditions the mutation has not affected pathogencity. However, if a reduced reaction occurs, it is necessary to determine whether the reduction in pathogenicity is attributable to the known deficiency or to some other change in the mutant line. If mutants for the same deficiency show like reductions in pathogenicity, the probability is enhanced that the reduction is attributable to the deficiency. Stronger evidence may be gained from crossing the mutant with a wild-type line and studying the pathogencity of progeny lines. If, in a sufficient test, the reduction in pathogenicity is confined to the lines that carry the mutant gene, it can be concluded that the latter conditions this reduction. The results of tests thus far made on the pathogenicity of progeny lines from such crosses are in Table 4.

Most of the vitamin deficiencies thus far studied had no perceptible effect on pathogenicity. The typical lesion reaction was incited by mutants deficient, respectively, for nicotinic acid, biotin, inositol, and pantothenic acid. However, no reaction greater than flecking was incited by the choline or the riboflavin deficient mutants tested. It seems clear from the evidence given in Table 4 that the reduction in pathogenicity of the choline mutants is attributable to the choline deficiency; it has not yet been similarly established that the riboflavin deficiency is accountable for reduced pathogenicity.

None of the nitrogen base deficient mutants so far studied incited symptoms more pronounced than flecking. The data in Table 4 give strong evidence that the losses in pathogenicity of the nitrogen base mutants there listed are attributable to the specific deficiencies shown. Similarly, none of the amino acid deficient mutants tested incited the lesion reaction, and in the cases studied genetically all the available evidence (Table 4) indicates that the loss in pathogenicity is attributable to the deficiencies concerned.

On the other hand, mutants deficient for reduced sulfur usually showed no loss in pathogenicity. Genetic studies have shown that morphological as well as biochemical mutation has occurred in each of the 2 reduced sulfur mutants shown in Table 3 that failed to incite lesions; the loss of pathogenicity in these cases may prove attributable to the morphological mutant genes.

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TABLE 3.—*Maximum symptoms incited by biochemical mutants derived from a wild-type line that incites the lesion reaction with the apple varieties employed*

Deficiency	Mutant No.	Number of tests[a]	Maximum symptom	Deficiency	Mutant No.	Number of tests[a]	Maximum symptom
Vitamins				Purines	2702	3	None
Nicotinic acid	2599	3	Lesion	Purines	2892	3	Fleck[b]
Biotin	2570	2	Lesion	Purines	2988	3	None
Inositol	1969	4	Lesion	Purines	3010	3	None
Inositol	2532	3	Lesion	Amino acids			
Inositol	2694	2	Lesion	Histidine	1261	4	Fleck[b]
Pantothenic acid	2584	3	Lesion	Histidine	1336	5	Fleck[b]
Pantothenic acid	3050	1	Lesion	Histidine	2112	3	Fleck[b]
Pantothenic acid	3053	2	Lesion	Proline	2366	3	None
Choline	1905	6	Fleck	Proline	3021	1	None
Choline	2383	3	Fleck[b]	Methionine	2602	3	None
Choline	2959	2	Fleck	Methionine	2781	1	None
Choline	2844	4	Fleck	Methionine	2978	1	Fleck[b]
Choline	2997	2	Fleck	Arginine	1119	5	None
Riboflavin	2343	4	Fleck[b]	Arginine	2180	2	None
Riboflavin	3115	1	None	Arginine	2386	3	None
Nitrogen bases				Arginine	2951	2	None
Pyrimidines	934	2	None	Arginine	2955	2	None
Pyrimidines	953	4	Fleck[b]	Arginine	2957	3	None
Pyrimidines	1907	4	None	Arginine	2993	1	None
Pyrimidines	2340	4	Fleck[b]	Arginine	2996	2	None
Pyrimidines	2393	3	None	Arginine	3000	3	None
Pyrimidines	2481	2	None	Arginine	3069	3	None
Pyrimidines	2784	4	Fleck[b]	Arginine	3072	3	None
Pyrimidines	2835	3	None	Reduced sulfur	1195	5	Lesion
Pyrimidines	2903	3	None	Reduced sulfur	1901	4	Lesion
Pyrimidines	3060	2	None	Reduced sulfur	1970	3	Fleck[b]
Purines	950	4	Fleck[b]	Reduced sulfur	2302	5	Lesion
Purines	2106	4	Fleck[b]	Reduced sulfur	2396	5	Lesion
Purines	2129	3	Fleck	Reduced sulfur	3013	2	Lesion
Purines	2317	5	None	Reduced sulfur	3025	2	Lesion
Purines	2334	3	None	Reduced sulfur	3026	3	None
Purines	2370	3	None	Reduced sulfur	3067	1	Lesion
Purines	2380	3	Fleck[b]	Reduced sulfur	2377	1	Lesion

[a] The several tests with a given mutant were made on different dates. Trees of 1–7 apple varieties were employed in each test.

[b] Flecks were very small or pit-like.

DISCUSSION.—The major portion of the planned work with morphological and color mutants has been completed, and the results have been prepared for more detailed publication elsewhere. Through this work methods were developed for inducing mutations in *V. inaequalis* and studying the mutant characters genetically, numerous marker characters were discovered, and progress was made in the development of linkage groups and chromosome maps. The chief purposes of this work were to contribute to knowledge of the genetics of this pathogen and to facilitate the studies that deal more directly with problems of physiology and pathology.

The work with biochemical mutants is in a comparatively early stage; consequently, discussion of it at this time will be brief. Many biochemical mutants with various categories of nutritional deficiencies have already been induced and characterized, and it seems probable that a wide variety of additional ones can be produced. It has been shown that in most of the biochemical mutants so far characterized the deficiencies are complete. In the genetic studies, these biochemical mutant characters have each segregated in 1:1 ratio, indicating single-gene control. The fact

TABLE 4.—*Tests made with progenies of certain biochemical mutants × normal to determine whether or not reduction in pathogenicity was correlated with nutritional deficiencies*[a]

Parent mutant No.	Deficiency	Number of tests with mutant progeny[b]	Number of tests with *normal* progeny[b]
1905	Choline	6	6
2383	Choline	4	2
953	Pyrimidines	4	4
1907	Pyrimidines	6	4
2340	Pyrimidines	3	2
2481	Pyrimidines	3	3
950	Purines	4	4
2106	Purines	3	3
2380	Purines	3	2
1119	Arginine	4	4
1261	Histidine	8	8
1336	Histidine	2	2

[a] In all cases the mutant progenies showed the reduced pathogenicity characteristic of the respective parent mutants, and the *normal* progenies showed no reduction in pathogenicity.

[b] Each test included one or both of the lines derived from a single chromatid. Corresponding progeny from the same asci were used for testing mutant and *normal* lines.

that in numerous cases genes at different loci have been shown to affect the requirement for the same metabolite makes possible a study of step-wise biochemical reactions. The effects of biochemical mutations on pathogenicity have been shown to range from no apparent change in some cases to complete suppression in others. These early findings encourage further studies aimed at elucidation of the mechanisms that determine these differences.

Experiments are in progress to determine whether reductions in pathogenicity of biochemical mutants can be compensated by furnishing the deficient materials to the fungus by applications made either externally to the host or through its tissues. Consideration is also being given to possibilities that microflora of the leaf surface or other factors in the leaf surface environment may contribute significant amounts of deficient substances in the case of biochemical mutants in which there appears to be no diminution of pathogenicity.

The results thus far obtained indicate that the ability of *V. inaequalis* to synthesize certain vitamins, nitrogen bases, and amino acids may be of critical importance to its pathogenicity. Whether similar nutritional deficiencies play a part in the differential pathogenicity of its wild-type lines to various host varieties and species remains to be determined. These investigations are being continued.

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DICHLONE—A COINED NAME FOR THE FUNGICIDAL CHEMICAL 2,3-DICHLORO 1,4-NAPHTHOQUINONE

The word *Dichlone* has been selected as a coined name for the fungicidal chemical 2,3-dichloro 1,4-naphthoquinone. This name has been approved by the Interdepartmental Committee on Pest Control. The American Chemical Society, the American Medical Association, and the American Phytopathological Society are agreeable to its use.

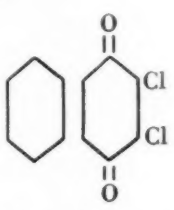
The Interdepartmental Committee on Pest Control secured the following information from the originator of this coined name but takes no responsibility for the contents thereof. Approval of the name *Dichlone* by the Interdepartmental Committee indicates that the name is available for free use in designating the pure chemical 2,3-dichloro 1,4-naphthoquinone.

Dichlone has been sold under the trade name, *Phygon*. The name *Dichlone* applies to the pure chemical and a technical grade shall indicate the percentage of *Dichlone* present.

Physical and Chemical Properties

Empirical Formula: $C_{10}H_4O_2Cl_2$

Structural Formula:



—Interdepartmental Committee on Pest Control, S. W. Simmons, Chairman, Morris Alpert, Secretary.

GLYODIN—A COINED NAME FOR THE FUNGICIDAL CHEMICAL 2-HEPTADECYL GLYOXALIDINE ACETATE

The term *Glyodin* has been coined to designate the fungicide, 2-heptadecyl glyoxalidine acetate. The use of this term has been approved by the Interdepartmental Committee on Pest Control, and has also been accepted by the American Chemical Society, the American Medical Association, and the American Phytopathological Society. The Interdepartmental Committee secured the following information from the originator of this coined term but takes no responsibility for the contents thereof.

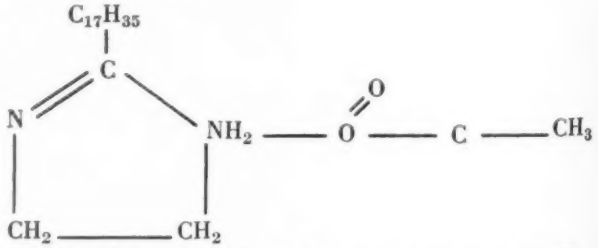
A commercial fungicide containing this compound is sold as Fruit Fungicide 341 under the trade-mark *Crag*.

Approval of the term *glyodin* by the Inter-departmental Committee on Pest Control indicates that the name may be used in designating the pure chemical, 2-heptadecyl glyoxalidine acetate.

Physical and Chemical Properties

Emperical Formula: $C_{22}H_{44}O_2N_2$

Structural Formula:



—Interdepartmental Committee on Pest Control, S. W. Simmons, Chairman, Morris Alpert, Secretary.

TWO MUSKMELON MOSAIC VIRUS STRAINS FROM CENTRAL FLORIDA [1]

C. W. Anderson

SUMMARY

Two strains of muskmelon mosaic virus induced systemic symptoms in 4 *Cucurbita* species, *Cyclanthera pedata*, and *Melothria pendula*. Both failed to invade 2 *Luffa* species systemically. The strains were distinguished from each other principally by their reactions in plants of watermelon, citron, *Lagenaria siceria*, 2 *Cucumis* species, and *Momordica charantia*. Neither strain was transmitted by *Myzus persicae* in limited tests. Both withstood 70°C./10 minutes and 14 days' aging in extracted sap. The latent strain withstood 1/5,000 dilution; the type, 1/10,000. The latent strain partially protected squash plants from systemic invasion by the type, but the latter showed no immunological relationship to watermelon mosaic virus in cucumber or watermelon plants. Environmental factors influenced the results of tests of the physical properties considerably. The type strain may not be identical with that described by Rader et al., but it is very closely allied to it and to the cantaloupe virus described from South Carolina by Aycock.

Muskmelon mosaic virus is differentiated from 3 other cucurbit viruses. Squash and citron plants proved useful for distinguishing among the strains of watermelon mosaic and muskmelon mosaic viruses and southern cucumber mosaic virus.

Muskmelon mosaic virus is uncommon in cucurbit plantings in central Florida although its latent strain is often found in *Melothria* plants.

Muskmelon mosaic virus (*Marmor melonis* Rader et al) (6) was described from New York in 1947. In 1952, Anderson (1) stated that 2 unidentified cucurbit viruses occur in Florida. This report indicates that both are strains of muskmelon mosaic virus, shows that measurements of the physical properties of the latter are altered by environmental factors, and extends the known host range of muskmelon mosaic virus within the Cucurbitaceae.

MATERIALS AND METHODS.—The viruses used were the cantaloupe strain (1) of muskmelon mosaic virus, obtained from northwestern Marion County near Williston in 1952; the latent (*Melothria*) strain (1) of this virus, obtained from Bradenton in 1951; the type strain of watermelon mosaic virus, *Marmor citrulli* Anderson (2); and an isolate representing the southern strain of cucumber mosaic virus (*Marmor cucumeris commelinae* H.).

The methods used were identical with those of the study of watermelon mosaic virus (2) unless otherwise noted. Some parallel inoculations were made with the 2 strains and each of the 23 cucurbitaceous types (13 species). Attempts were made to recover each strain from noninoculated parts of each cucurbitaceous plant type. Often, separate attempts were made to recover virus from inoculated portions of the plants. At least 21 days elapsed between inoculation and attempted recovery in all cases in which systemic symptoms did not develop in the test plants. As before (2), systemic symptoms usually developed in the first or second leaf formed following inoculation. However, there were some notable exceptions to this rule in the case of the muskmelon mosaic virus strains.

RESULTS.—*Hosts and symptoms.*—Both virus strains induced systemic symptoms in and were recovered from plants of 8 varieties of *Cucurbita pepo melopepo* Alef. and *C. pepo medullosa* Alef.; 3 varieties of *C. moschata* Duchesne; *C. maxima* Duchesne; *C. okeechobeensis* (Small) Bailey; *Cyclanthera pedata* Schrad.; and *Melothria pendula* L. Both strains evidently caused only local lesions in plants of *Luffa cylindrica* Roem. and *L. aegyptiaca* Mill. Only the type strain induced systemic symptoms in plants of *Cucumis sativus* L. (cucumber), *C. melo* L. (Hale's best cantaloupe), *Citrullus vulgaris citroides* Bailey (Colorado preserving citron), *Lagenaria siceria* Standl., and *Momordica charantia* L.; and only the type strain induced local lesions in plants of *Citrullus vulgaris* Schrad. (Cannon ball watermelon). The latent strain caused only local lesions in plants of *Lagenaria siceria*, and possibly caused faint systemic symptoms in plants of *Momordica charantia*.

With 1 exception, each strain was readily recovered on Early prolific straight-neck squash (*Cucurbita pepo melopepo* Alef.) plants from each plant type in which systemic symptoms were induced. Neither strain was recovered from noninoculated portions of *Momordica* plants, although both strains were recovered with difficulty from the inoculated parts. The type strain was recovered from the inoculated portions of each plant type in which it caused only local lesions, but not from the noninoculated parts of these plants. The latent strain was readily recovered from the inoculated portions of cucumber and cantaloupe plants. It was less readily recovered from the noninoculated parts of these plants and from the inoculated portions of *Lagenaria* plants. All other attempts to recover virus from plants of the above species failed. Neither strain induced symptoms in nor was recovered from plants of the non-cucurbitaceous species *Nicotiana tabacum* L., *Capsicum frutescens* L., or *Zinnia elegans* Jacq.

The symptoms induced by both strains were more severe in winter than in summer. The latent strain induced symptoms similar to but less severe than those

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of the type. Rader et al (6) described the symptoms caused by *M. melonis* in certain plants. In this study, the type strain caused only chlorotic rings in *Momordica* plants. It caused mottling and severe shoestring in *Cyclanthera* plants; the distorted leaves often resembled tendrils. It caused small brown necrotic local lesions, similar systemic spots, and extremely severe stunt, distortion, chlorosis, and mottle in *Lagenaria* plants. *Cyclanthera* often developed several leaves before the systemic symptoms of either strain appeared. The latent strain induced few symptoms other than mild mottling in *Cyclanthera* plants. Both strains tended to induce ring patterns in plants of the *Cucurbita* species. Both caused projections at the ends of the leaf veins and enations on the under surfaces of the leaves in plants of certain of the *Cucurbita* species in winter. Projections were most common in the cultivated forms of *C. pepo;* enations, in *C. moschata* and *C. maxima.* Symptoms were least evident in *C. okeechobeensis.* The type strain caused symptoms in cantaloupe plants similar to those illustrated by Rader et al (6) in winter. In summer, it caused little distortion, relatively few projections, and a mottle somewhat resembling that shown by Anderson (2) as caused by the type strain of watermelon mosaic virus in the same cantaloupe variety.

Recovery of both strains from only the inoculated portions of *Momordica* plants despite the fact that the type strain induced systemic symptoms in them could indicate that a virus complex was present. However, neither strain was readily recoverable from the inoculated portions of *Momordica* plants. Plants displaying the highest tolerances in tests of physical properties were the ones saved for future inoculum, while the latent strain proved more readily recoverable from inoculated than from noninoculated parts of cucumber and cantaloupe plants. It was once recovered from a symptomless but inoculated *Cyclanthera* plant. Since there was no evidence of contamination and no virus was ever recovered from a control plant—although this was attempted many times—it is most logical to suppose that the concentration of the type strain had not built up to the point at which it would have been recoverable from noninoculated portions of *Momordica* plants, and that no complex was involved. The fact that the type strain recovered from noninoculated parts of plants such as citron and *Lagenaria* was unchanged from the original isolate and distinct from the latent strain in the original respects indicates that the type strain symptoms do not result from a combination of the latent strain with some other virus, since the latent strain does not invade citron or *Lagenaria* plants systemically.

Physical properties.—The thermal death points of both strains lay between 70 and 75°C./10 minutes. The tolerance to dilution of the latent strain lay between 1/5,000 and 1/10,000. Its resistance to aging lay between 21 and 28 days at 18–21°C. It retained its infectivity in frozen sap (−17°C.) for 385 days, the longest period tested.

The tolerance to dilution of the type strain lay between 1/10,000 and 1/50,000. Its resistance to aging lay between 14 and 21 days. Tests of the 2 strains were made under similar but not identical environmental conditions. The type strain retained its infectivity in frozen sap (−17°C.) for 288 days, the longest period tested.

Environmental factors strongly influenced the measurements of the physical properties of the 2 strains. For instance, the type strain withstood 60°C./10 minutes but not 65°C. in summer, although it withstood 70°C. in winter. The latent strain barely withstood 1/500 dilution in summer but survived 1/5,000 dilution in winter.

Aphid transmission tests.—Aphids (*Myzus persicae* Sulzer) were fasted for 12–25 hours, given an acquisition penetration period of 18–30 seconds on diseased straight-neck squash or *Melothria* plants, and transferred to healthy crook-neck or straight-neck squash plants for 25–55 minutes, using 1 aphid per test plant. The aphids did not infect 30 squash plants (total = 60 plants) with either strain. Attempts to use long acquisition feeding periods failed because *M. persicae* did not feed well on the source plants.

Although the tests were limited, the failure of *M. persicae* to transmit the muskmelon mosaic virus strains is noteworthy in view of the fact that this aphid infected 15 of 51 squash plants with watermelon mosaic virus (2) in similar tests. Moreover, Freitag (5) obtained 2 aphid-transmissible viruses (other than cucumber mosaic virus) and 3 beetle-transmissible viruses from cucurbits in California. Evidently, those viruses which he transmitted by aphids were not transmissible by beetles and vice versa.

Immunological studies.—Watermelon plants systemically invaded by the type strain of watermelon mosaic virus developed about 45 per cent as many local lesions when inoculated with the type strain of muskmelon mosaic virus as did plants not infected with watermelon mosaic virus. Cucumber plants systemically invaded by the type strain of muskmelon mosaic virus readily developed symptoms of the type strain of watermelon mosaic virus when inoculated with that virus, and both viruses were recovered on watermelon plants.

Straight-neck squash plants systemically invaded by the latent strain of muskmelon mosaic virus developed no additional symptoms in 17–20 days following inoculation with the type strain, although healthy plants of the same age developed symptoms of the type strain in 5–8 days. Attempts to recover virus from noninoculated portions of these plants on watermelon and citron plants indicated that the type strain was present in all 6 squash plants inoculated with that strain only. The type strain was also recovered on both watermelon and citron from 2 of 6 squash plants inoculated with the latent strain and later with the type. The 2 watermelon plants which developed lesions following inoculation with sap from squash plants inoculated with both strains developed 8 lesions/leaf. The 6 plants inocu-

lated with sap from squash plants inoculated only with the type strain developed about 21.2 lesions/leaf. Citron plants inoculated with sap from squash plants inoculated with the latent strain and later with the type developed systemic symptoms of the type strain 1–3 days later than did plants inoculated with sap from squash plants inoculated only with the type strain. Although the latent strain did not completely protect squash plants against systemic invasion by the type, partial protection was secured.

DISCUSSION.—*Relationships between the 2 strains of muskmelon mosaic virus.*—The type and latent strains resemble each other strikingly in the symptoms they induce in all tested forms of *Cucurbita* (13 varieties, 4 species: *C. pepo, C. maxima, C. moschata, C. okeechobeensis*). They also resemble each other in the symptoms they cause in several other hosts; in their physical properties; and in their failure to prove transmissible by *Myzus persicae*. Only the type strain induced symptoms in watermelon and citron plants; systemic symptoms in *Lagenaria* plants; and definite symptoms in *Momordica* and *Luffa aegyptiaca* plants. The 3 non-cucurbitaceous species (*Nicotiana tabacum, Capsicum frutescens,* and *Zinnia elegans*) were immune from both strains. The latent strain partially protected squash plants from systemic invasion by the type. Therefore, the latent and type strains are strains of 1 (muskmelon mosaic) virus.

Relationships between the latent and type strains and other viruses.—Only 4 viruses need be compared in detail with the 2 strains described here, for reasons previously explained (2). These viruses (2, Table 1) are: cucurbit mosaic virus (*Marmor astrictum* H.), watermelon mosaic virus (*M. citrulli* Anderson), muskmelon mosaic virus (*M. melonis* Rader et al), and bottle gourd mosaic virus (*Cucumis* virus 3 Vasudeva & Lal). The 2 strains described here differ strikingly from *M. astrictum* in physical properties and in causing symptoms in plants of *Cucurbita pepo*. They differ from *M. citrulli* in physical properties, in not proving transmissible by *Myzus persicae*, in their immunological reactions, and in the reactions they cause in various cucurbitaceous plants including citron, watermelon, and the *Cucurbita* species. They differ from *Cucumis* virus 3 in physical properties and in the reactions they cause in watermelon and *Momordica* plants. Additional differences can be found between the 2 strains described here and the 3 viruses discussed above, and the strains described here are believed unrelated to them.

The 2 strains also differ from *M. melonis* in their physical properties. However, they resemble *M. melonis* more than they resemble the other 3 viruses. The physical properties of *M. melonis* were studied in melon rather than in squash. Possibly, greater similarities could be found if the 2 viruses were tested in the same host. Moreover, the thermal death point of *M. melonis* and of the cantaloupe ("type") strain from Florida both lie between 60 and 65°C./10 minutes if tests of the Florida cantaloupe isolate are conducted during the summer. The latent strain was less tolerant of dilution than *M. melonis* in tests performed in summer, but both Florida strains withstood greater dilution than *M. melonis* in tests conducted in winter. Therefore, the Florida strains cannot be considered distinct from *M. melonis* on the basis of the reported differences in physical properties. The 2 Florida strains and *M. melonis* are the only viruses considered which do not invade watermelon plants systemically. The latent strain clearly differs from the type strain of *M. melonis*, since the latent strain induces no symptoms in cucumber and cantaloupe plants. The cantaloupe ("type") strain from Florida may also be distinct, since it causes local lesions in watermelon plants. The similarities in host range and in the symptoms induced by *M. melonis* in muskmelon and cucumber plants and those caused by the Florida cantaloupe strain in cantaloupe and cucumber plants indicate that the 2 Florida strains are strains of *M. melonis* Rader et al. The varietal name *obscurum* is applied to the latent strain from Florida. The Florida cantaloupe ("type") strain is so closely related to *M. melonis* that a varietal name is not desirable.

Anderson (1) stated that the Florida cantaloupe ("type") strain resembled the cantaloupe mosaic virus reported from South Carolina by Aycock (3, 4). The symptoms (4) caused by Aycock's virus in squash, watermelon, and cucumber plants, particularly, are very similar to those caused by the Florida "type" strain in these plants. Aycock's virus also induces symptoms in cantaloupe plants which resemble those caused by the Florida "type" strain in some respects. The thermal death point of Aycock's virus lies between 60 and 65°C. It is therefore nearly identical with that of *M. melonis*, and identical with that of the Florida "type" strain in summer tests. The slightly greater severity of the lesions which Aycock's virus caused in watermelon plants and other minor differences between his virus and the Florida "type" strain may result from differences in environmental conditions, which strongly affect the reactions of the Florida "type" strain, and from differences in the plant varieties used. Possibly, the Florida "type" strain resembles Aycock's virus more closely than that of Rader et al, since the South Carolina and Florida viruses both cause local lesions in watermelon plants.

Technical description.

Marmor melonis Rader et al var. *obscurum* var. nov.

Common name: Latent muskmelon mosaic virus.

Origin: *Melothria pendula* from Bradenton, Florida, December, 1951.

Host range: Infects all tested cucurbits at least locally, including *Cucumis sativus, Lagenaria siceria, Momordica charantia,* and *Cucurbita pepo,* except the tested forms of *Citrullus vulgaris* and possibly *Luffa aegyptiaca. Nicotiana tabacum* immune.

Differential symptoms: Symptoms similar to those of *M. melonis* in *Cucurbita* spp., *Melothria pendula,* and *Cyclanthera pedata,* but usually milder. Causes no symptoms in *Cucumis sativus, C. melo,* and possibly

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Momordica charantia, and has difficulty in invading these species systemically or fails to do so. Induces only local lesions in *Lagenaria siceria*, *Luffa cylindrica*, and possibly *L. aegyptiaca*, and fails to invade them systemically.

Transmission methods: Similar to *M. melonis*.

Physical properties (determined in *Cucurbita pepo melopepo*): Similar to *M. melonis*. Thermal death point, between 70 and 75°C./10 minutes; tolerance to dilution, between 1/5,000 and 1/10,000; resistance to aging, between 21 and 28 days (18–21°C.). Resistance in tests of physical properties lower when tests performed during summer.

Immunological reactions: Partially protects squash plants against systemic invasion by *M. melonis*.

Test plants.—Of the plants tested, squash (*Cucurbita pepo melopepo* var. Early prolific straight-neck) and Colorado preserving citron (*Citrullus vulgaris citroides*) are among the most valuable for quick identification of the 2 muskmelon mosaic strains and the strains of watermelon mosaic virus. Parallel inoculations between *Marmor cucumeris commelinae* H. and the type strain of muskmelon mosaic virus were made in citron. Only cucumber mosaic virus caused local lesions on inoculated citron leaves, and the systemic symptoms of each virus type were distinctive. Cucumber mosaic virus was recovered from noninoculated parts of citron plants to tobacco. Cucumber mosaic virus had difficulty in invading citron and causing symptoms in it during the summer.

Importance of muskmelon mosaic virus.—Rader et al (6) and/or Aycock (4) indicate that *M. melonis* is common and seedborne, but not very damaging. They noticed fruit symptoms only in squash. The virus is rare in commercial plantings in Florida (1), but its latent strain is common in plants of *Melothria pendula* in Alachua County. Field symptoms in *Melothria* plants are variable and sometimes nearly masked. Several factors—including the possibilities of partial masking of symptoms in summer, seed transmission in *Melothria*, and the rarity of the non-aphid vectors in commercial plantings—may explain why the virus is rare in commercial cucurbit plantings in central Florida but common in the perennial *Melothria* plants.

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REACTION OF LYCOPERSICON SPECIES TO REGIONAL ISOLATES OF SEPTORIA LYCOPERSICI [1]

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SUMMARY

Lycopersicon accessions representing 5 species, 3 sub-species, and a series of suspected interspecific crosses were inoculated with 8 regional isolates of the tomato leafspot fungus, *Septoria lycopersici*. Disease was evaluated by several criteria of which diameter of lesions was found most consistent and reliable for evaluating relative resistance of accessions and relative virulence of isolates of the pathogen.

The isolates differed significantly in virulence and could be separated into 3 groups in order of increasing virulence: 1) South Dakota (original and sub-isolate); 2) Canada, South Carolina (original) and Indiana (sub-isolate); 3) Wisconsin, Indiana (original), and South Carolina (sub-isolate).

The tomato leafspot fungus, *Septoria lycopersici* Speg., under suitable environment, incites a serious defoliation disease. Most attempts to incorporate resistance to it from other species of *Lycopersicon* into an acceptable variety of *L. esculentum* Mill. have not been immediately successful, and there has not been complete agreement on the disease resistance qualities of various accessions. Locke (7) reported 3 accessions, P.I. 126449, P.I. 127827, and P.I. 134418, to be quite resistant while Lincoln and Cummins (6) designated these same accessions susceptible. Accession P.I. 128285 was only slightly defoliated in the field according to Butler (4) but Alexander et al (1) found it susceptible. The 2 *L. pimpinellifolium* accessions (P.I. 126925 and P.I. 126952), considered by Locke to be resistant, were rated moderately resistant

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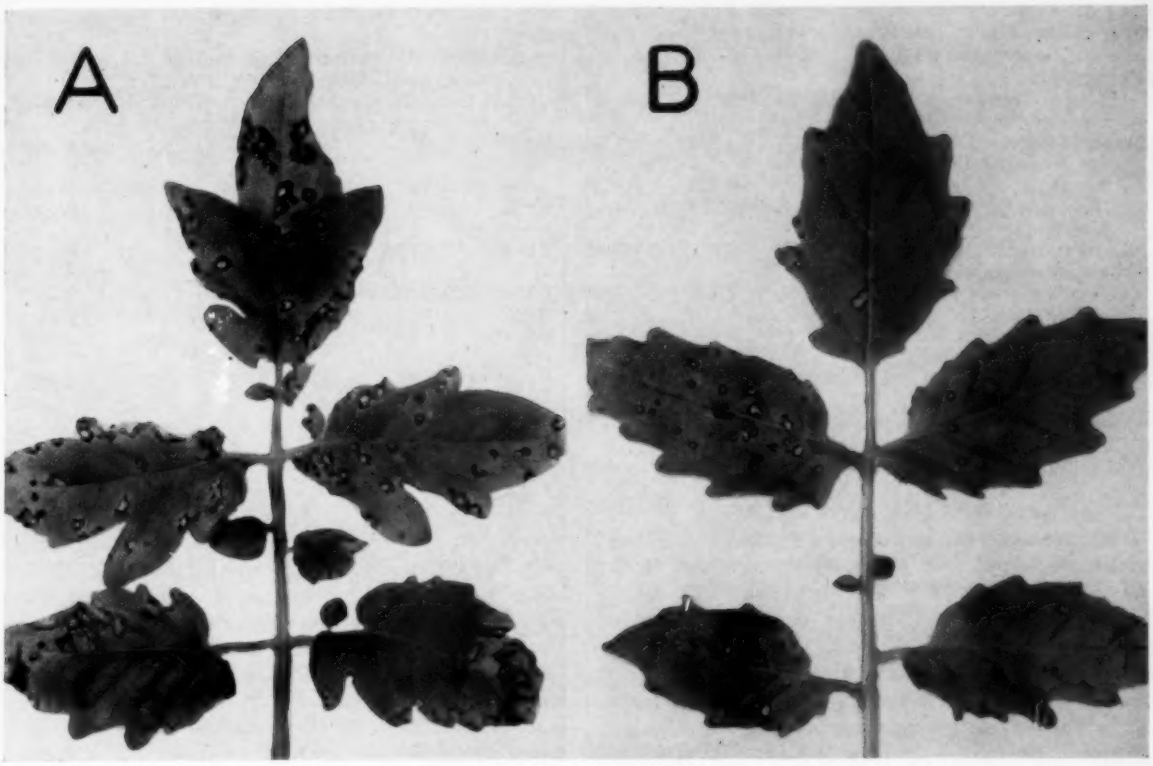

FIG. 1. Lesions produced by the South Carolina isolate of *S. lycopersici* 14 days after inoculation. A) Foremost (check); B) P. I. 126443.

and susceptible, respectively, by Alexander et al. There is some agreement, however, between the greenhouse observations of Andrus and Reynard (3) and the field ratings of Alexander et al.

One possible reason for these inconsistent reports may be the practice among investigators to use local isolates of the pathogen. Levin (5) used different isolates, but concluded that his isolate in Argentina was the same as those in this country. MacNeill (8) noted differences in pathogenicity between 2 strains of the fungus.

The present study was undertaken to compare the performance of 5 isolates and 3 sub-isolates of the leafspot fungus on accessions of 5 *Lycopersicon* species (*L. hirsutum*, *L. peruvianum*, *L. pimpinellifolium*, *L. glandulosum*, and *L. esculentum*), 3 subspecies (*L. hirsutum* f. *glabratum*, *L. esculentum* var. *cerasiforme*, and *L. peruvianum* var. *dentatum*), and a series of progenies suspected to be from the cross *L. esculentum* × *L. peruvianum* (2).

METHODS AND MATERIALS.—Seeds of the tomato accessions were obtained from the North Central Regional Plant Introduction Station at Ames, Iowa. These were planted in a sand-soil mixture in greenhouse flats, and were transplanted 3–4 weeks later. Seven rows were transplanted across the shorter dimension of each flat, ½ row being allotted to each accession and 1 full row to a commercial variety as check.

Inoculum was prepared by seeding sterile Petri plates of 2 per cent potato-dextrose-agar with 1–2 ml. of a concentrated suspension of spores. Incubation of these cultures for 2–3 weeks at room temperature resulted in abundant sporulation in most cultures. At inoculation time, when the plants were 5–6 weeks old, the spores were dislodged into sterile distilled water and further dispersed in a Waring Blendor for about 1 minute. Approximately 1 plate culture was used to inoculate each flat and the inoculum was applied to the foliage with a Hudson Continuous sprayer. Only 1 isolate was used in each experiment. All flats were immediately placed in a moist chamber and allowed to remain there for 48 hours, after which they were returned to a bench in the greenhouse held at 68–75°F. Good disease development usually appeared within 13–15 days (Fig. 1). Observations at this time were recorded to establish the pattern of disease in individual accessions for each of the regional isolates of the fungus.[2] All trials were conducted between October 7, 1952 and April 6, 1953.

[2] Acknowledgment is given Drs. C. F. Andrus, Vegetable Breeding Laboratory, Charleston, South Carolina, G. S. Pound, University of Wisconsin, Madison, Wisconsin, M. L. Tomes, Purdue University, Lafayette, Indiana, and L. T. Richardson, University of Western Ontario, London, Ontario, for supplying cultures from their particular regions.

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TABLE 1. *Correlation coefficients between average diameter of 25 representative lesions produced by 8 regional isolates of the tomato leafspot fungus*

Species and number of accessions compared		Coefficients with isolates								
		S.D.:Can.	S.D.:Wis.	S.D.:S.C.	S.D.:Ind.	Can.:Wis.	Can.:S.C.	Can.:Ind.	Wis.:S.C.	Wis.:Ind.
L. esculentum (and var. *cerasiforme*)	(14-16)	0.983[a]	0.968[a]	0.970[a]	0.970[a]	0.987[a]	0.986[a]	0.986[a]	0.972[a]	0.991[a]
L. hirsutum	(3-4)	0.899	0.929	0.963[b]	——	0.985[b]	0.978[b]	——	0.994[a]	——
L. hirsutum f. *glabratum*	(3-4)	——	0.997[b]	——	0.990	0.993	——	0.988	——	0.996[a]
L. peruvianum (and var. *dentatum*)	(25-30)	0.942[a]	0.932[a]	0.967[a]	0.945[a]	0.907[a]	0.948[a]	0.931[a]	0.961[a]	0.847[a]
L. pimpinellifolium	(24-33)	0.963[a]	0.973[a]	0.975[a]	0.969[a]	0.967[a]	0.981[a]	0.955[a]	0.966[a]	0.961[a]
L. glandulosum	(4-7)	0.965[a]	0.978[a]	0.973[a]	0.946[a]	0.991[a]	0.969[a]	0.925[b]	0.987[b]	0.939[b]
L. esculentum × *L. pimpinellifolium*	(35-54)	0.971[a]	0.972[a]	0.961[a]	0.959[a]	0.985[a]	0.987[a]	0.985[a]	0.975[a]	0.990[a]
L. esculentum (cml. var.)	(9-12)	0.995[a]	0.977[a]	0.993[a]	0.990[a]	0.989[a]	0.993[a]	0.994[a]	0.990[a]	0.987[a]

[a] Significant at 1 per cent level.
[b] Significant at 5 per cent level.

RESULTS.—*Number of Lesions Per Plant.*—The total number of lesions per plant was determined in the first 2 experiments, but this procedure was discontinued thereafter. The number of lesions produced following artificial inoculation varied considerably between experiments. Correlation coefficients between the Wisconsin and Indiana isolates of the average number of lesions per plant were significant (5 per cent level) in only 3 of 7 comparisons. Correlations between the average number of lesions produced and the average green weight of infected leaves proved significant in 10 of 12 analyses, 9 at the 1 per cent level.

Average Diameter of Lesions.—The diameters of 25 representative lesions were recorded for each accession in each experiment to determine 1) the size of lesions on the different accessions and 2) the size of lesions produced by the fungus isolates. Correlations between all isolates indicated that the size of lesions on the various accessions usually varied in a similar manner for all isolates (Table 1). A variance analysis of the data for 42 selected accessions revealed significant differences in over-all reaction to the individual isolates (Table 2).

Correlations between the average diameter of 25 representative lesions and the average number of lesions per plant proved significant in only 9 of 15 instances, 7 of these being at the 1 per cent level.

DISCUSSION.—The reports of previous investigators who have studied the reaction of *Lycopersicon* accessions to the leafspot fungus have not been in complete agreement, as previously mentioned. Locke suggested that the variability of the material tested under the same accession numbers by different investigators was a contributing factor to these divergent results. It does not seem probable, however, that such variability could be entirely responsible for different workers obtaining contradictory data concerning accessions within entire species. In the above mentioned experiments, the species *L. hirsutum* exhibited decided resistance to the leafspot fungus, but the mean lesion diameter for this species, in comparison with the others tested, was not of the same order as determined by Locke. The mean lesion diameters for both *L. glandulosum* and *L. peruvianum* were less than for *L. hirsutum*, and all 3 species gave a smaller mean lesion diameter than *L. esculentum*.

With regard to the reaction of individual accessions, P.I. 127827 was found to be susceptible as indicated by Lincoln and Cummins rather than decidedly resistant as according to Locke. P.I. 128650, listed as susceptible by Lincoln, and Cummins, was considered resistant by the author.

The inconsistent results of different investigators obtained using material of the same accession numbers may be due, in part, to variability within these accessions. However, differences in pathogenic virulence of isolates of the causual organism may also be a contributing factor. Such variation in pathogenic capabilities of disease producing organisms, often demonstrated but not always of significance in disease control, should be recognized in evaluating and utilizing resistance.

TABLE 2. *Average diameter in mm. of 25 representative lesions produced by 8 regional isolates of the tomato leafspot fungus on 42 selected Lycopersicon accessions.*

Isolates[a]							
S.D.	Can.	Wis.	S.C.	Ind.	S.D.[b]	Ind.[c]	S.C.[d]
0.74	1.06	1.22	1.13	1.24	0.83	1.13	1.24

[a] L.S.D.: 5 per cent—0.12; 1 per cent—0.15.
[b, c, d] Sub-isolates of original South Dakota, Indiana, and South Carolina isolates, respectively.

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STUDIES ON ANTIBIOTIC SOIL ORGANISMS. II. BACTERIA AND FUNGI ANTAGONISTIC TO PYTHIUM ARRHENOMANES IN SUGARCANE SOILS OF LOUISIANA [1]

H. H. Luke and T. D. Connell

SUMMARY

This study reports the results of a survey of fungi and bacteria in sugarcane soils of Louisana, in regard to the antagonistic activity exhibited by these organisms toward *Pythium arrhenomanes*, the causal agent of sugarcane root rot. During this study approximately 5,500 bacterial isolates and 5,000 fungal isolates were tested in artificial culture for antagonism to the root rot fungus. Approximately 3.6 per cent of the bacteria and 16.0 per cent of the fungi tested exhibited antibiotic activity toward the parasite. In respect to the zones of inhibition produced, fungi were more inhibitory than were bacteria. Size of bacterial and fungal populations was affected by soil reaction, the largest bacterial population being obtained from soils with pH values above 7.6, whereas the largest fungal populations occurred in soils with an acid reaction. The highest fungal antibiotic activity was obtained from soils in which root rot was most severe whereas the antibiotic activity exhibited by bacteria was considerably higher in soils where root rot was not severe.

In a preceding article[2] the results of a survey of actinomycetes in Louisiana sugarcane soils and their antagonistic activity toward *Pythium arrhenomanes* Dresch., a causal agent of sugarcane root rot, were reported. It was also reported that in various experiments 18.5–31.5 per cent of the actinomycetes tested were antagonistic to the root rot pathogen. As the antibiotic activity of the actinomycetes was greater in soils in which the root rot was the least severe, there was a possibility that other soil microorganisms might also be important factors in checking the growth and pathogenicity of the pythium fungus. The present study was consequently designed to gain information concerning the antagonistic effects of fungi and bacteria in Louisiana sugarcane soils on the root rot pathogen.

Materials and methods.—Soil samples were taken, at 3-month intervals over a period of 1 year, from 13 localities in the sugarcane producing area of Louisiana. These samples were from the following soil types: 1) light soil from the Mississippi River area (Mahoon very fine sandy loam); 2) heavy soil from the same area (Sharkey clay); 3) soil from the Red River area (Yahola fine sandy loam); 4) First Terrace soils near Lafayette (Olivier silt loam complexes); 5) soils from the Bayou Teche area (Jeanerette and Baldwin silt loams).

After moisture and pH determinations were made, each soil sample was diluted with sterile distilled water and the bacterial and fungal populations calculated using the standard dilution culture methods described by Thornton[3] and Martin[4]. Dilutions of 1:10,000 and 1:1,000,000 were used to determine fungal and bacterial populations respectively. From 7 to 10 dilution cultures were made from each soil sample. These were tested for antagonism to *P. arrhenomanes* according to a technique described by Cooper and Chilton[2] which consisted of streaking the isolate to be tested on one side of a petri plate containing Czapek's agar medium and planting pythium on the opposite side. These plates were then incubated for 7 days, at 29°C. for bacterial isolates or at 23°C. for fungal isolates, and the zones of inhibition (the distance between the

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The authors express their appreciation to Dr. S. J. P. Chilton for suggestions and criticisms during the course of this study.

[2] Cooper, W. E., and S. J. P. Chilton. 1950. Studies on antibiotic soil organisms. I. Actinomycetes antibiotic to Pythium arrhenomanes in sugar-cane soils of Louisiana. Phytopathology 40: 544-552.

[3] Thornton, H. G. 1922. On the development of a standardized agar medium for counting soil bacteria, with special regard to the repression of spreading colonies. Ann. Appl. Biol. 9: 241-274.

[4] Martin, J. P. 1950. Use of acid, rose bengal and streptomycin in the plate method of estimating soil fungi. Soil Sci. 69: 215-232.

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TABLE 1.—*Seasonal variation in antibiotic values from isolates from various sugarcane areas*

Area	Summer		Fall		Winter		Spring	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
Red River	765	164	2340	82	1479	97	13337	64
Teche	6907	108	3015	178	3880	104	9987	207
First Terrace	3157	80	6421	106	4070	132	19067	186
Mississippi River light	2755	208	2691	232	3278	158	5458	118
Mississippi River heavy	2446	190	3093	352	3151	296	4858	283
Average	3205	150	3512	190	3172	157	10549	172

foremost edge of the *Pythium* mycelium and the edge of the bacterial or fungal isolate) were measured. The "antibiotic value" as defined by Cooper and Chilton[2] was used as a quantitative measure of the antibiotic activity of the organisms in the various soils. This value was calculated by multiplying the "antibiotic index" (mean width of zone of inhibition) by the number of thousands of bacteria or fungi per gram of soil.

SEASONAL VARIATION OF ANTIBIOTIC VALUES.—Soil collections were made during the summer and fall of 1951 and the winter and spring of 1952. The bacterial populations, calculated from the number of colonies appearing in the dilution cultures, ranged from 9,250,000 to 102,500,000 per gram of soil, while the range in fungal populations was 53,000 to 444,000 per gram. The antibiotic values of the organisms obtained from these soil samples are presented in Table 1.

The average antibiotic value exhibited by bacterial isolates obtained in the spring was markedly greater than that found in other seasons. Furthermore, this greater value occurred in all 5 sugarcane areas although it was most pronounced in the sandy and silt loams from the Red River, Teche and First Terrace areas. Fungal isolates did not exhibit any definite seasonal trends in their antibiotic activity; but the antibiotic values of this group were consistently higher for heavy soils (Sharkey clay) than for other soil types tested.

RELATION OF SOIL REACTION TO ANTIBIOTIC ACTIVITY AND POPULATIONS.—Soil samples tested during the investigation showed a wide range in pH values. In order to study the relation between soil reaction, bacterial and fungal populations, and antibiotic activity, pH values which ranged from 5.5 to 8.0 were divided into 5 classes (each class having an interval of 0.5). Data obtained from this study are in Table 2. In general, larger bacterial populations were found in soil samples with pH values above 7.5 than in those with an acid reaction; however, the reverse was true regarding fungal isolates. Bacterial antibiotic values were erratic and showed no definite trend, with the 2 highest antibiotic values occurring at the highest and lowest pH ranges. The antibiotic values exhibited by fungi followed a more regular pattern with the highest values occurring at pH 6.1–7.0 and progressively lower values occurring with increasing acidity or alkalinity.

DEGREE OF INHIBITION EXHIBITED BY THE ISOLATES TESTED.—To determine the percentage of isolates exhibiting various degrees of antagonism to the root rot organism, the bacterial and fungal isolates were grouped in classes according to the width of the zone of inhibition. These data presented in Table 3 show that approximately 3.6 per cent of the bacteria and 16.0 per cent of the fungi tested were antagonistic to *P. arrhenomanes*. These data also indicated that the bacteria were rather weakly antagonistic since 68.8 per cent of the antagonistic bacteria produced inhibition zones of 5 mm. or less and only 2.5 per cent produced zones wider than 10 mm. More than 60 per cent of the antagonistic fungal isolates produced zones of inhibition more than 5 mm. in width and 21.9 per cent produced zones wider than 10 mm.

THE RELATION OF NUMBERS AND ANTIBIOTIC ACTIVITY OF ISOLATES TO ROOT ROT SEVERITY.—Observations made over a period of more than 20 years have indicated that damage to sugarcane caused by pythium root rot has been slight in some areas included in this survey and severe in others. The severity of root rot found in the various areas together with the numbers and antibiotic values of the bacteria and fungi from those areas are in Table 4. In general, lower numbers of bacteria occurred in soils with moderate to severe root rot, whereas the largest populations were obtained from soils exhibiting slight root rot. An opposite trend occurred with respect to fungal populations, the largest numbers being obtained from the areas in which root rot was most severe. Data representing the relation between root rot severity and antibiotic activity showed patterns similar to and somewhat more definite than those exhibited by the bacterial and fungal populations. The lowest antibiotic value exhibited by bacteria was obtained from soils in which root rot was severe, while the highest antibiotic values were

TABLE 2.—*The relation of soil reaction to numbers and antibiotic values of bacteria and fungi*

pH class	Number of organisms per gram of soil (thousands)		Antibiotic value	
	Bacteria	Fungi	Bacteria	Fungi
5.5-6.0	26520	176	6094	128
6.1-6.5	24220	158	3742	208
6.6-7.0	28430	176	3952	231
7.1-7.5	29560	125	4767	177
7.6-8.0	44090	104	7901	130

TABLE 3.—*Degree of antagonism to Pythium arrhenomanes exhibited by bacterial and fungal isolates*

Organism	Number of isolates		Number of isolates in each inhibition class				
	Tested	Antagonistic	0 mm.	1-5 mm.	6-10 mm.	10-16 mm	16 mm
Bacteria	5683	202	5481	137	60	0	5
Fungi	5218	816	4402	317	320	155	24

obtained from soils in which root rot was not a serious disease. Fungal antibiotic values exhibited a contrary trend, the lowest values occurring in soils where root rot was not a problem whereas the highest values were obtained from soils in which the disease was moderate to severe.

CONCLUSIONS.—During this survey a total of 5,648 bacterial and 5,218 fungal isolates from Louisiana sugarcane soils were tested for antagonism to *P. arrhenomanes*. Approximately 3.6 per cent of the bacteria and 16.0 per cent of the fungi tested were antagonistic to this root parasite in artificial culture. Bacterial antagonists produced zones of inhibition which varied from a trace to 27 mm. and in general were less inhibitory than were fungal antagonists which produced zones ranging from 1 to 58 mm.

TABLE 4.—*The relation of Pythium root rot severity to the antibiotic activity and numbers of bacteria and fungi from various sugarcaue areas*

Collection area	Root rot severity	Thousands of organisms per gram of soil		Antibiotic value	
		Bacteria	Fungi	Bacteria	Fungi
Red River	Slight	27460	95	4490	102
First Terrace	Slight	36570	180	8178	126
Teche	Moderate	29390	142	5946	150
Mississippi River light	Moderate	19960	131	3545	179
Mississippi River heavy	Severe	24800	197	3387	280

The antibiotic values obtained from the various sugarcane areas indicated that fungi did not exhibit any material seasonal fluctuation in their antibiotic activity; however, bacteria exhibited greater antibiotic activity during the spring. The size of bacterial and fungal populations was affected by soil reaction. Bacterial populations obtained from soils with pH values above 7.6 were materially larger than from soil samples with pH values below this figure. Fungal populations were larger in soils having an acid reaction than in those with pH values above 7.0. The optimum pH for fungal antibiotic activity was approximately 6.9.

The highest fungal antibiotic activity as estimated by antibiotic values was obtained from soils in which root rot was most severe. Bacterial antibiotic activity, however, was considerably higher in soils where root rot was not a problem than in soils in which the disease was severe. These results, coupled with the fact that antibiotic values for bacteria were much larger than those for fungi, suggest that antagonistic bacteria are more important than fungi as factors affecting root rot severity.

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VAPOR ACTION OF CERTAIN MERCURY SEED TREATMENT MATERIALS[1]

D. C. Arny and Curt Leben

SUMMARY

The vapor action of 4 mercury seed treatment materials varied greatly. The 2 materials with the greatest vapor action, Ceresan M and Panogen, were able to control the *H. victoriae* seedling blight of oats by vapor action alone. Of the 2, Ceresan M acted more slowly.

In the course of our studies with the organic mercury seed treatment materials it was found that certain of these preparations were consistently more effective than others for the control of various seed-borne diseases of small grains. Since vapor action is generally acknowledged for this group of chemicals and has been demonstrated for some of the older materials (3, 7), it seemed of value to study the correlation between vapor action of some of the newer materials and their effectiveness for disease control. Of particular interest was the investigation of the blight of Vicland oats (C.I. 3611) incited by *Helminthosporium victoriae* M. & M., a fungus that is carried from one season to the next by means of the seed[2] and under some conditions in the soil. The fungus appears to be carried beneath the hulls of the seed to some extent and perhaps in the other layers of the caryopsis itself. We are not aware of histological evidence as to the exact position of the fungus.

A short account of the present work has already appeared (2).

MATERIALS AND METHODS.—For yield tests under field conditions seed treatment materials were applied at the recommended rate[3] directly to seed naturally carrying *H. victoriae* and thoroughly mixed as in ordinary treatment. Treated seed was stored in kraft paper bags for at least 2 weeks before planting. In order to minimize infection by the pathogen from the soil, plantings were made in fields where oats had not been grown for several years. Four replications of 3-row plots 1 rod long were used. Yield data are based on the center row of each plot.

Vapor action was studied by enclosing the seed with the material to be tested in an air-tight container for a given period of time, taking care that the seed and test material were not in direct contact. Two dusts, Agrox (6.7 per cent phenyl mercury urea) and Ceresan M (7.7 per cent ethyl mercury *p*-toluene sulfonanilide), and 2 liquids, Panogen (2.2 per cent methyl mercury dicyan diamide) and Setrete (7.0 per cent phenyl mercuric ammonium acetate), were examined. The container was a glazed crock with a capacity of approximately 500 gm. of oats. The test fungicide was spread in an inverted Coplin jar top, which was placed on the bottom of the crock. Five to 7 gm. of Vicland oat seed, naturally carrying *H. victoriae*, was scattered on the bottom of the crock around the jar top. The amount of test material used per crock was 0.48 gm. of Agrox or Ceresan M, 0.77 ml. of Panogen, or 0.51 ml. of Setrete.[3] A glass cover was sealed to the crock with petroleum jelly. A separate crock was used for each interval of exposure. Trials made at different times served as replications. The temperature during the exposure period was 20–25°C.

The uptake of fungicidal vapor by seed was detected by means of an agar diffusion biossay of exposed seed, and by the reduction in the incidence of blighted seedlings in greenhouse tests. The bioassay, which used *Glomerella cingulata* (Stonem.) Spauld. & v. Schrenk as the indicator fungus, has been described for Ceresan M (1), although in the present work large plates (8) rather than Petri dishes were used. It was found that Agrox, Panogen, and Setrete on seed also could be assayed reliably by means of this method. The procedures were the same as for Ceresan M except that for a given material the amount on an unknown sample was determined by reference to a dosage-response curve based on the response of seeds carrying known amounts of that same material. The greenhouse tests were made in soil in the same manner as previously described for this disease (6); no evidence was found that the soil carried *H. victoriae*.

EXPERIMENTAL RESULTS.—*Field tests.*—Since severe Victoria blight reduces yield considerably, one measure of blight control is the yield of plants from seed treated with various test chemicals. Representative yield data with the 4 seed treatment materials are in Table 1.

The Ceresan M and Panogen treatments resulted in significant increases in yield, whereas no increase was noted with Agrox or Setrete.

Vapor action studies.—The bioassay results from 3 trials are summarized in Fig. 1. Panogen proved to possess the greatest vapor action. Tests made on caryopses from which the hulls had been removed after exposure showed that part of the Panogen vapor penetrated beneath the hulls. Ceresan M vapor was

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[2] The term "seed" as applied to oats in this paper includes the caryopsis and the adhering hulls (lemma and palea).

[3] On the basis of a full crock of seed (500 gm.) these amounts are equivalent to the recommended rates: 0.5 oz./bu. for Agrox and Ceresan M, 0.75 fl. oz./bu. for Panogen, and 0.5 fl. oz./bu. for Setrete.

TABLE 1.—*Yield of Victland oats when seed carrying H. victoriae was treated by the direct application of certain seed treatment materials*

Material	Average yield in bu./acre		
	1952	1953	Ave.
Ceresan M	50.6	74.2	62.4
Panogen	45.9	74.2	60.1
Agrox	37.3	62.6	49.9
Setrete	35.6	62.6	49.1
None	36.1	65.4	50.8
L.S.D., 5%	6.7	6.1	4.5

taken up much more slowly and did not reach nearly so high a concentration. Though not illustrated in Fig. 1, removal of the hulls after exposure indicated that part of the Ceresan M vapor also penetrated beneath the hulls. The vapor action of Agrox and Setrete was much less than for the other 2 materials: even after 32 days, their activity on the seed was equivalent to less than 0.05 oz. per bushel.

It will be noted from Fig. 1 that the amount of Panogen vapor taken up by the seed reached the equivalent of 3 oz. of Panogen per bushel in 16 days, some 4 times the amount that would have been on the seed had the material been applied directly to a whole crock of seed. When the seed assaying 3 oz. per bu. was planted in soil, characteristic mercury injury was apparent in the resulting seedlings as might be expected with such a high concentration of this material.

The vapors from Ceresan M and Panogen were effective in controlling the seedling blight of oats in the greenhouse (Table 2). Panogen gave adequate control after an exposure of 1 day, whereas Ceresan M required an exposure of more than 8 days for comparable results. The vapors from Agrox or Setrete were much less effective even after 32 days.

A study was made of the relation of the amount of seed in the crock to the uptake of Panogen vapor. Various amounts (from 7.5 to 480 gm.) of seed were placed in crocks. In each crock, 0.77 ml. of Panogen was placed in a Coplin jar cover that was placed on top of the seed. Crocks were sealed as before. There were 3 replicate crocks for each amount of seed. Seed from the surface layer was bioassayed after 16 days. The results (Fig. 2) indicate that as the amount of seed was increased up to 120 gm. (a layer about 1.5 cm. deep) the amount of vapor taken up by the seed in the surface layer decreased. In 2 other experiments, crocks were filled with seed, and seed from various depths assayed after 16 days. Fungicide detected at the several depths was as follows: surface—0.4 oz. per bu., 1 cm. down—0.2 oz. per bu., 2 cm.—0.1 oz. per bu., 3 cm.—trace, and 4 cm. and below—none. It is of interset to note in this connection that Thomas (11) found that formaldehyde vapor did not penetrate through 1.3 cm. of seed of certain ornamental plants.

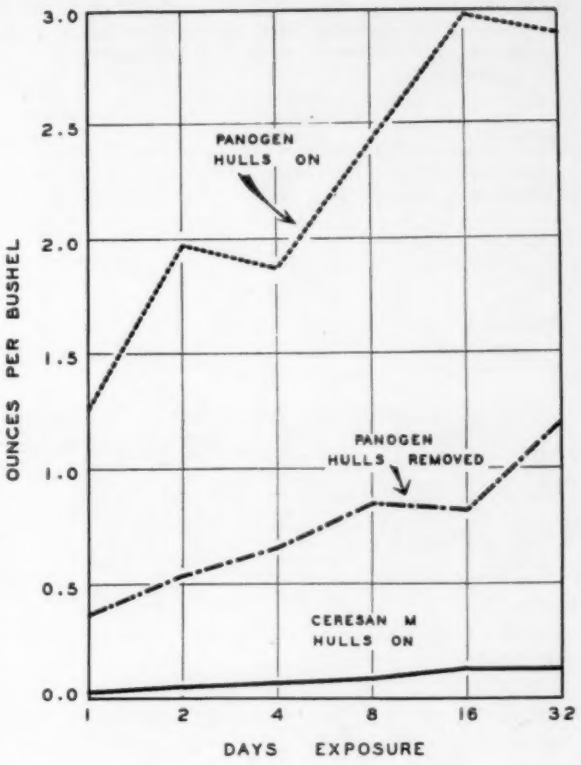

FIG. 1. The amount of fungicide on seed when seed was exposed to Ceresan M or Panogen vapors for various times in sealed crocks. Determinations made by bioassay of seed in the surface layer and expressed in terms of ounces per bushel. After the exposure period, hulls were removed from some seeds and only the caryopses assayed.

DISCUSSION.—In the present studies there was a strong parallel between the degree of vapor activity of 4 mercury materials and their effectiveness for the control of the Victoria blight when applied directly to the seed as in the usual seed treatment procedure. It was shown that the vapors from Ceresan M and Panogen, materials with strong vapor action, are capable of penetrating beneath the hulls of oat seed. This penetrating property no doubt enhances the effectiveness of these materials, particularly for the control of pathogens that may be found beneath the hulls of the seed. Furthermore, it appears that because of vapor action, complete coverage of the seed during the treating procedure is not essential for control of Victoria blight of oats. In studying cotton seed treatment Lehman (7) reached a similar conclusion. Thus vapor action is probably an important characteristic for effective coverage for the control of similar seed-borne pathogens of these and other crops. It is concluded from the present data that the effectiveness of Ceresan M and Panogen for the control of the Victoria blight is due in large part to the vapor action of these materials, and that the ineffectiveness of

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TABLE 2.—*Percentage of blighted seedlings from seed carrying H. victoriae after exposure to vapors from several seed treatment materials in sealed crocks*

Material	Time seed exposed to vapor	Seedling blight[a]
	(days)	(per cent)
Ceresan M	1	16
	2	9
	4	8
	8	6
	16	1
	32	1
Panogen	1	2
	2	3
	4	1
	8	2
	16	1
	32	0
Agrox	8	26
	32	10
Setrete	8	25
	32	19
None	—	20

[a] Average of 2 greenhouse trials.

Agrox and Setrete may be attributed to a proportionate lack of this property.

The findings of the present work substantiate those of Lehman (7), who in 1943 noted that several organic mercury materials then in use emitted fungicidal vapors. When cotton seed, carrying disease inciting fungi, were exposed to vapors from ethyl mercury materials and then germinated, significant reductions in the numbers of blighted seedlings were observed. Seed decay, caused by fungi not usually regarded as important pathogens on cotton seedlings, was also reduced by vapor action. One material, shown to have little or no vapor action, was observed to be less effective than those with strong vapor action when used for disease control in field tests.

Gassner (3) tested vapor action on the germination of bunt spores. The phenyl mercury compounds he used were not active across an air space, although they did inhibit the spores when in direct contact. On the other hand, he noted that the alkyl mercury materials, especially ethyl- and methyl-, were highly active across an air space.

The present work may offer a logical explanation for data obtained by other investigators. Koehler and Bever (5), in working with oat smut, found that if seed was stored only 1 day before planting, Panogen treatment gave complete control, whereas Ceresan M treatment did not; however, if seed was planted 1 or 4 weeks after treatment both Panogen and Ceresan M were effective. Hansing (4) studied the effectiveness of Agrox, Ceresan M, Panogen, and Setrete in relation to the length of time treated seed was stored before planting. For the control of oat smut a shorter storage period was needed for Panogen-treated seed than for seed treated with Agrox, Ceresan M, or Setrete. Ceresan M and Panogen were comparable for the control of Victoria blight, but a longer storage

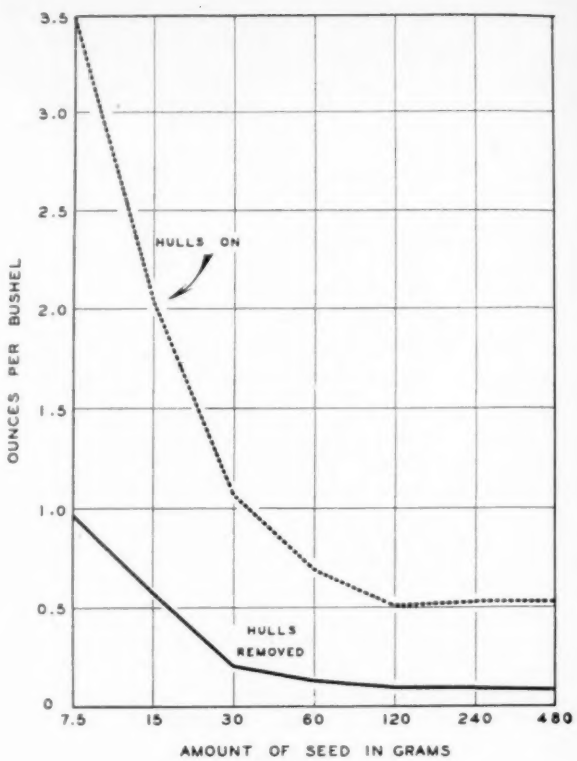

FIG. 2. The amount of fungicide on seed when various quantities of seed were exposed to Panogen vapor for 16 days in sealed crocks. Determinations made by bioassay of seed in the surface layer and results expressed in terms of ounces per bushel. After the exposure period, hulls were removed from some seeds and only the caryopses assayed.

period was required for Ceresan M. It is believed that the relative rate and the amount of vapor action of these materials could well explain the results of these workers. Vapor action was probably important in the experiments of Meiners (9) and Meiners and Dietz (10), who tested the same 4 materials for the control of head smut of mountain brome grass. With direct application they found that Ceresan M and Panogen gave adequate control, whereas Agrox and Setrete did not.

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PHYTOPATHOLOGICAL NOTES

Varietal Reaction to Ceratostomella Fimbriata in Sweet Potato[1]. W. J. Martin. The recent publication by Cheo[2] reporting high resistance to black rot among sweet potato varieties and seedlings has stimulated great interest in the possibility of combatting black rot through the use of resistant varieties. Therefore, it was considered desirable to test under the conditions of inoculum and climate prevailing in Louisiana those varieties and seedlings which were reported as highly resistant.

The sweet potato varieties and seedlings used in this study, and considered highly resistant to black rot by Cheo, included Allgold (Oklahoma 24), Sunnyside (B-5944), Kandee (K-1716), B-6097 and B-5941. Unit 1 Porto Rico, which is the principal commercial variety in Louisiana, and O-123, a Louisiana seedling, also were used.

Two isolates of *Ceratostomella fimbriata* were used in the tests. Isolate "A" was obtained by the writer from a diseased root of the Unit 1 Porto Rico variety grown at St. Francisville, Louisiana. Isolate "B" was obtained through the courtesy of Pen Ching Cheo from Beltsville, Maryland.

Fig. 1. Section of a root of the Allgold variety, upper, and of the Unit 1 Porto Rico variety of sweet potato showing depth of penetration of black rot fungus 21 days after inoculation.

Inoculations were made at 3 equidistant points on each root by a puncture method which was essentially

[1] The varieties and seedlings used in this study were furnished by C. E. Steinbauer, Plant Industry Station, Beltsville, Maryland, and J. J. Mikell, Department of Horticultural Research, Louisiana State University, Baton Rouge, Louisiana.

[2] Cheo, Pen Ching. 1953. Varietal differences in susceptibility of sweet potato to black rot fungus. Phytopathology **43**: 78-81.

Table 1.—*The mean*[a] *diameter in millimeters of black rot lesions induced by artifically inoculating*[b] *3 sweet potato varieties with 2 isolates of Ceratostomella fimbriata under 2 temperatures*

Variety or Seedling	Mean Diameter in Millimeters of Lesions at 21°C.		Room Temperature (25-30°C.)	
	Isolate "A"	Isolate "B"	Isolate "A"	Isolate "B"
Unit 1 Porto Rico	32.50	34.75	40.50	40.75
Allgold	33.50	29.50	32.75	11.50
O-123	32.25	30.00	31.25	30.25
L.S.D. 5%	n. s.[c]	n. s.	n. s.	9.81
1%	n. s.	n. s.	n. s.	13.87

[a] Mean of 12 lesions (3 lesions on each of 4 roots).
[b] Inoculated on September 26, 1953; notes on October 23, 1953.
[c] The n. s. means not statistically significant.

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TABLE 2.—*The mean*[a] *diameter in millimeters of black rot le ons induced by artificially inoculating*[b] *6 sweet potato varieties with 2 isolates of Ceratostomella fimbriata under 2 temperatures*

Variety or Seedling	Mean Diameter in Millimeters of Lesions at 21° C.		Room Temperature (23-28° C.)	
	Isolate "A"	Isolate "B"	Isolate "A"	Isolate "B"
Unit 1 Porto Rico	23.50	27.00	24.75	28.75
Allgold	19.25	18.75	18.25	20.00
Sunnyside	18.50	12.75	21.75	13.75
Kandee	18.75	18.00	16.50	19.25
B-6097	13.00	13.25	13.50	14.00
B-5941	22.75	15.25	25.25	23.25
L. S. D. 5%	n. s.[c]	6.33	5.01	5.76
1%	n. s.	8.60	6.80	7.81

[a] Mean of 12 lesions (3 lesions on each of 4 roots).
[b] Inoculated on November 24, 1953; notes on December 16, 1953.
[c] The n. s. means not statistically significant.

the same as that described by Cheo. In all tests, 4 roots of each variety were inoculated with each isolate of the fungus. After inoculation the roots in each replicate were placed in large evaporating dishes lined on the bottom with moist filter paper.

Results of the experiment are in Tables 1 and 2. Diameter of lesions resulting from the inoculations was used as the criterion of varietal reaction. The results in Table 1 show that differences among varieties were not significant with either isolate at 21°C. However, at room temperature, differences among varieties were highly significant in the experiment where isolate "B" was used. Comparative penetration of the fungus in the varieties Unit 1 Porto Rico and Allgold at 21°C. with isolate "A" is illustrated in Fig. 1.

From the data in Table 2 it is apparent that differences among the 6 varieties were not significant with isolate "A" at 21°C., and that differences were significant with isolate "B" at 21°C. At room temperature with isolate "B" the lesions on B-5941 were not different from those on Unit 1 Porto Rico.

It is apparent from the results of this study that the reaction of a sweet potato variety or seedling to the black rot fungus depends upon the specific parasitic race of the fungus to which it is exposed, as well as upon the temperature conditions under which the disease develops. Seedling B-6097 showed good tolerance to black rot under the conditions of these experiments. —Department of Plant Pathology, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

Peronospora in Radish Roots.[1] G. B. Ramsey, M. A. Smith and W. R. Wright.[2] Although the downy mildew fungus (*Peronospora parasitica*) occasionally is found causing discoloration of cauliflower curds, cabbage heads[3] and turnip roots[4] on the market, it is uncommon on the roots of radishes or has not been recognized. However, discoloration of radish roots by *Peronospora* caused serious loss of radishes of the red globe type grown in the market-garden district south of Chicago during October, 1953. Furthermore, topped radishes prepackaged in polyethylene bags at shipping points in Texas and Florida during February and March, 1954 showed similar discoloration on the Chicago market.

The diseased radishes showed brown to black discolorations of the epidermis generally about half-way down the sides of the roots. The discoloration had no particular pattern; sometimes there was an elongated streak, but usually the discoloration was blotchy and frequently spread around the circumference of the roots. Many of the roots having this discoloration were smooth, but often there was a slight russeting of the epidermal tissue and some cracking (Fig. 1A). The internal flesh of the roots was firm and grayish-brown to black (Fig. 1B). This discoloration is similar to that found in turnip roots. However, in turnips the fungus is usually conspicuous in the crown and from that region continues invasion of the central part of the root, whereas in these radishes there was no discoloration at the crown to indicate spread of the fungus from that region. Infections were so commonly found ¼–½ in. below the crown that they suggested the possibility of the roots being infected by spores washed down from the leaves during the latter part of the growing season.

On diseased radish roots kept at room temperature (60-72° F.) in a moist chamber the fungus produced conidiospores in abundance within 2 days. The spores germinated readily within 2 hours when placed in water (Fig. 1C). Spores in a water suspension were placed on the sides of 10 healthy radish roots kept in a moist chamber at room temperature. Infection took

[1] Investigation conducted by the Biological Sciences Branch, Agricultural Marketing Service, U. S. Department of Agriculture, in cooperation with the Department of Botany, University of Chicago.
[2] Principal Pathologist, Pathologist, and Assistant Pathologist respectively, A.M.S., U.S.D.A.

[3] Ramsey, G. B. 1935. Peronospora in storage cabbage. Phytopathology 25: 955-957.
[4] Gardner, M. W. 1920. Peronospora in Turnip roots. Phytopathology 10: 321-322.

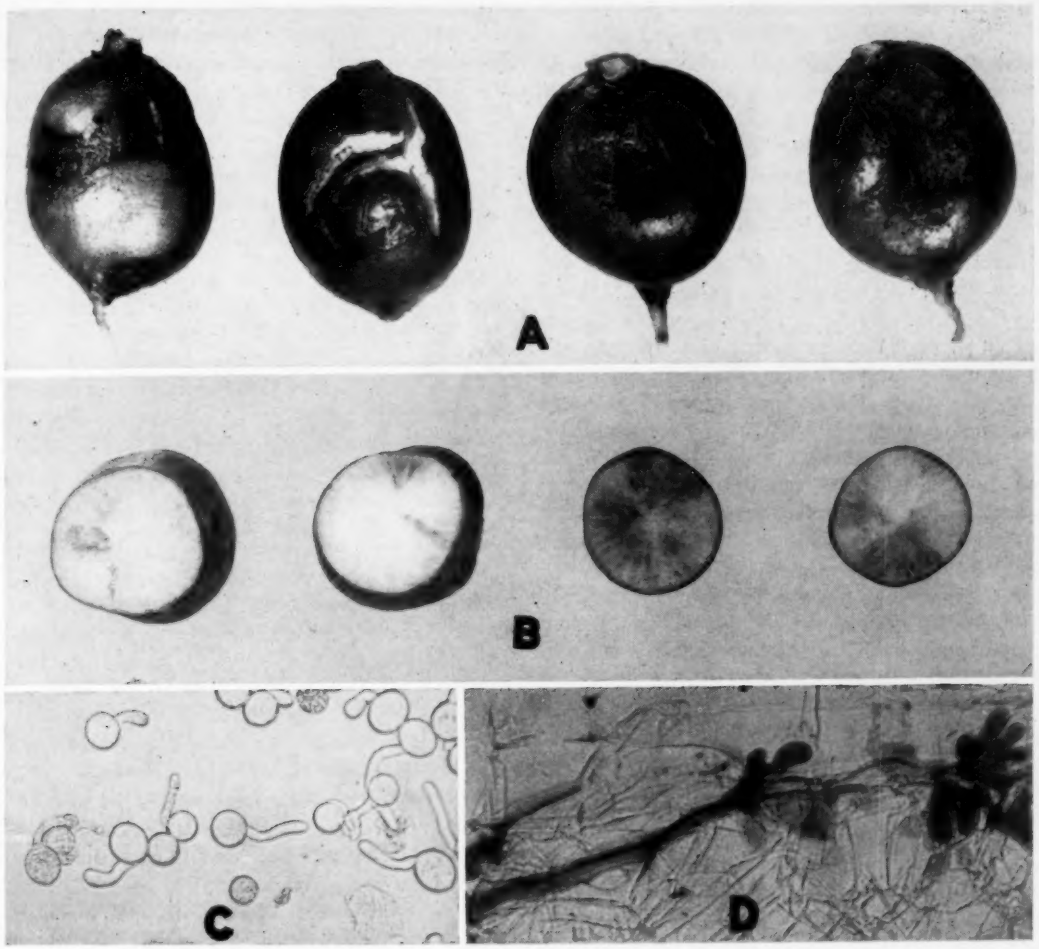

FIG. 1. Peronospora in radish roots. A) External view of affected roots. B) Internal discoloration of roots. C) Spores germinated in water in 2 hours. D) Hyphae and haustoria within affected tissues.

place in the inoculated radishes through the uninjured epidermis in 8 of the roots within a week. Six healthy radishes used as controls in the same moist chamber remained free of disease. It appears, therefore, that radish roots could become infected by spores washing down the sides of the roots while they are still in the soil. This type of infection would be similar to potato tuber infections in the soil by *Phytophthora* spores from blighted vines.[5] The results of the above experiment and others of a similar nature also indicate the possibility that some of the discoloration found in prepackaged radishes may have resulted from germination of spores that contaminated the roots during the harvesting and bagging process. The polyethylene bags make an effective moist chamber for the damp radish roots when they are packed.

To check on infections through wounds, spores in a water suspension were placed on freshly cut surfaces of several radish roots that were kept in a moist chamber at room temperature. A slight darkening of the cut surfaces indicating that infection had occurred was visible within 3 days, and after 5 days the fungus was found to have penetrated the roots to a depth of ¼ in. Sections of epidermal and internal tissues of radish roots discolored by natural infections as well as by artificial inoculations showed extensive invasion by hyphae and the development of haustoria typical of *Peronospora* (Fig. 1D).—U.S. Department of Agriculture, Agricultural Marketing Service, Chicago, Illinois.

[5] Jones, L. R., N. J. Giddings, and B. F. Lutman. 1912. Investigations of the potato fungus Phytophthora infestans. Vt. Agr. Exp. Sta. Bul. 168.

Sporulation by the Brown Spot Fungus on Longleaf Pine Needles. BERCH W. HENRY.[1] The brown spot needle blight, caused by *Scirrhia acicola* (Dearn.) Siggers, continues to be a major obstacle to the

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satisfactory regeneration of longleaf pine (*Pinus palustris* Mill.) throughout much of its natural range. The premature death of a high proportion of the needle tissue, as a result of the disease, greatly reduces early height growth of longleaf pine and causes some mortality of seedlings.[2,3] The disease may also be of importance on loblolly pine (*P. taeda* L.)[4] and the decline of a plantation of ponderosa pine (*P. ponderosa* Laws.) in Missouri has been attributed largely to it.[5]

The pycnidial stroma of *S. acicola* are produced the year round on the brown spots and necrotic distal portion of diseased needles.[2,6] Masses of conidia are extruded in a viscid matrix during wet weather and are disseminated mainly by rain splash and to a slight extent by wind-driven rain drops.[2,7] Limited trials have indicated that the conidia are pathogenic[2,4,8]. Local diseasespread of the type that would be expected from rain-carried conidia is often evident on longleaf pine seedlings in nursery beds and sometimes on new needles of longleaf seedlings along the margins of recently burned areas. Prescribed burning is the accepted means of controlling brown spot on longleaf pine seedlings in the field.[2,3,9]

The occasionally rapid reinfection of relatively large burned areas and the not unusual occurrence of the disease on needles of large trees 50 ft. or more above ground suggest that *S. acicola* may be carried by air currents as well as by rain. Siggers[10] suggested that the ascospores of the fungus were wind-disseminated and responsible for infections high above the ground. Wolf and Barbour[6] showed that the ascospores were forcibly ejected from the ascocarps. Ascospores have been reported as occurring only in winter and spring[2,6] and then only after death of most of the needle[2]. It thus appeared that the role of the ascospores of *S. acicola* in the epidemiology of the brown spot disease needed further study. As a beginning, an attempt was made to determine the frequency of ascospore occurrence on longleaf pine needles in nature.

[2] Siggers, P. V. 1944. The brown-spot needle blight of pine seedlings. U. S. Dept. Agr. Tech. Bul. 870.

[3] Wakeley, P. C., and H. H. Muntz. 1947. Effect of prescribed burning on height growth of longleaf pine. Jour. Forestry 45: 503-508.

[4] Boyce, J. S., Jr. 1952. A needle blight of loblolly pine caused by the brown-spot fungus. Jour. Forestry 50: 686-687.

[5] Luttrell, E. S. 1949. Scirrhia acicola, Phaeocryptopus pinastri, and Lophodermium pinastri associated with the decline of ponderosa pine in Missouri. U. S. Dept. Agr. Pl. Dis. Reptr. 33: 397-401.

[6] Wolf, F. A., and W. J. Barbour. 1941. Brown-spot needle disease of pines. Phytopathology 31: 61-74.

[7] Verrall, A. F. 1936. The dissemination of Septoria acicola and the effect of grass fires on it in pine needles. Phytopathology 26: 1021-1024.

[8] Hedgcock, G. G. 1929. Septoria acicola and the brown-spot disease of pine needles. Phytopathology 19: 993-999.

[9] Siggers, P. V. 1934. Observations on the influence of fire on the brown-spot needle blight of longleaf pine seedlings. Jour. Forestry 32: 556-562.

[10] Siggers, P. V. 1939. Scirrhia acicola (Dearn.), n. comb., the perfect stage of the fungus causing the brown-spot needle blight of pines. Phytopathology 29: 1076-1077.

TABLE. 1.—Sporulation of *Scirrhia acicola* on longleaf pine needles through a 13-month period

Date of collection	Conidia	Ascospores; occurrence and condition of needles			
		1951 foliage		1952 foliage	
		dead	partly dead	dead	partly dead
Jan. 1952	+[a]	−[a]	−	0[a]	0
Feb. 1952	+	+	+	0	0
Mar. 1952	+	+	+	0	0
Apr. 1952	+	+	−	0	0
May 1952	+	+	+	0	−[b]
June 1952	+	+	+	0	+
July 1952	+	0[c]	0[c]	0	+
Aug. 1952	+	0	0	0	+
Sept. 1952	+	0	0	−	+
Oct. 1952	+	0	0	0	+
Nov. 1952	+	0	0	−	+
Dec. 1952	+	0	0	+	+
Jan. 1953	+	0	0	0	+

[a] Plus designates found, minus not found, and zero not examined microscopically. Only morphologically mature spores were recorded.

[b] Stromata first observed on 1952 foliage.

[c] All 1951 foliage was dead by July 11.

At least 6 brown-spot diseased needles were collected from each of 5 longleaf pine seedlings (planted in February 1949 on the Harrison Experimental Forest, Saucier, Miss.) at approximately biweekly intervals during the 13-month period of January 1952 to January 1953, inclusive. All collected needles were examined macroscopically and selected stromata were hand-sectioned and examined microscopically for the presence of conidia and ascospores of *S. acicola*. The resulting data are in Table 1.

Conidia were found throughout the 13-month period, thus confirming earlier reports that they are produced at all seasons of the year. They were found on the localized brown spots on living needles, on dead distal portions, and on completely dead needles.

Ascospores were found every month for the consecutive 12 months of February 1952 through January 1953. They were found on dead needles and necrotic distal portions of needles. In ⅔ of the latter cases the needles were dead for less than ½ their length. They were not found on the localized brown spots on living needles.

Thus the occurrence of morphologically mature ascospores of *S. acicola* the year-round on attached dead and partly dead longleaf foliage makes the possibility of their playing a role in the epidemiology of the brown spot disease appear more likely. Evidence on the quantity produced, means and extent of dissemination, and infectivity of the ascospores at different seasons will determine the importance of their role.—Harrison Experimental Forest, Saucier, Miss.

Miticidal Action of Barium and Manganese Ethylene Bisdithiocarbamates. SAUL RICH. Fungicides with miticidal properties have an obvious advantage over those which are not miticidal. This has become particularly true since the widespread use of DDT has aggravated the problem of phytophagous mites on crops. At least 2 organic fungicides which have become commercially available within recent years, have been helped in their development by the fact that they were also miticides. 2-Heptadecyl-2-imidazoline, described as a fungicide by Wellman and McCallan[1] in 1946, was later found by Chandler and Thurston[2] to be an effective killer of mites. The other material, 2,4-dinitro-6-caprylphenyl crotonate, was first described as a fungicide and miticide by Rich and Horsfall[3] in 1949.

In 1953, one of the compounds tested by the author for the control of apple scab was barium ethylenebisdithiocarbamate (hereinafter called BEBD). A technical grade of this compound, formulated as a wettable powder[4], was applied by hydraulic sprayer at the rates of ½ and 2 lb. of formulation per 100 gal., and using a pressure of 300 lbs. per sq. in. The treatments were tested on McIntosh apples by the half-tree technique[5]. A total of 6 sprays were made on the following dates: April 9, April 22, May 4, May 15, June 1, and June 16. DDT, 2 lb. per 100 gal., was applied as a separate treatment on May 20. Average maximum and minimum temperatures (°F.) during the experiment were: for April, 57 and 40; for May, 69 and 50; and for June, 81 and 56.

By July 2, the unsprayed checks averaged 43 per cent of their leaf area covered by scab whereas the foliage receiving the low and high dosages of BEBD had 5 per cent and 1.3 per cent scabby leaf area respectively. There was no noticeable spray injury on the foliage at either dosage of BEBD[6].

During the first week in July there was a heavy infestation in the orchard of European red mite (*Paratetranychus pilosus* C & F.). It was noted that those half-trees receiving the high dosage of BEBD remained decidedly greener than the trees around them. The difference appeared to be in amount of mite damage. As the mites are migratory, counts of cast skins were made on July 8, to measure relative densities of the mite population. Unsprayed foliage averaged 140 cast skins per sq. in., while none could be found on the foliage sprayed with the high dose of BEBD.

Subsequently, greenhouse tests[7] were made of the miticidal effectiveness of BEBD and of its 2 parent compounds, barium chloride and nabam (Dithane D-14). Freshly prepared BEBD was also tested to see if there was any peculiarity of the wettable powder which enhanced miticidal activity. Finally these materials were compared as miticides to manganese ethylenebisdithiocarbamate (Manzate), zineb (Dithane Z-78), 2-heptadecyl-2-imidazoline (Crag 341-C), and 2,4-dinitro-6-caprylphenylcrotonate (Iscothan). Named in parenthesis are the commercial formulations actually used in these tests. All materials were applied at a formulation concentration of 1:400, and included 1:2500 of Triton B-1956. Treatments were atomized onto bean plants heavily infested with the 2-spotted mite (*Tetranychus bimaculatus* Harvey). The mites and eggs were examined 1 week after spraying.

Under the conditions of the test, nabam, zineb, and barium chloride were entirely ineffective as miticides. The following materials completely killed the adults and nymphs: BEBD wettable powder, BEBD freshly prepared, manganese ethylenebisdithiocarbamate, 2-heptadecyl-2-imidazoline, and 2,4-dinitro-6-caprylphenyl crotonate. Only the last named material was highly toxic to the mite eggs in these trials.

All the materials except nabam were tested at concentrations equal to or higher than those used commercially. The nabam formulation at 1/400 is equivalent to 1 qt. per 100 gal., or ½ to ⅔ the concentration usually mixed with zinc sulfate commercially. It was found that preparations of BEBD and manganese ethylenebisdithiocarbamate required a wetting agent, Triton B-1956, in order to exert their miticidal effect.

Nabam, now rarely used as a fungicide without the addition of a metallic salt, was reported by Stoddard, et al.[8], to be very effective against *Tetranychus bimaculatus* H. on strawberries and carnations. They used a 0.15 per cent solution of Dithane D-14 plus Triton B-1956. This concentration is 60 per cent of that found to be non-miticidal in the present study. The apparent discrepancy indicates either a) the mite population in the present study is resistant to nabam, or b) the present formulation of Dithane D-14 is in some way different from that used by Stoddard, et al.

In summary, both barium and manganese ethylenebisdithiocarbamate were found to be effective miticides. Zineb and nabam proved to be ineffective. Barium ethylenebisdithiocarbamate was also found to be an excellent foliage protectant against apple scab.—Department of Plant Pathology and Botany, The Connecticut Agricultural Experiment Station, New Haven, Conn.

[1] Wellman, R. H., and S. E. A. McCallan. 1946. Glyoxalidine derivatives as foliage fungicides. 1. Laboratory studies. Contr. Boyce-Thompson Inst. 14: 151-160.

[2] Chandler, W. A., and H. W. Thurston, Jr. 1949. The constituents of "341" as apple scab fungicides. Phytopathology 39: 4.

[3] Rich, S., and J. G. Horsfall. 1949. Fungicidal activity of dinitrocaprylphenylcrotonate. Phytopathology 39: 19.

[4] The author is indebted to the Rohm and Haas Co., Philadelphia, Pa., for furnishing this material.

[5] Rich, S. 1952. Using half-tree plots for increasing the efficiency of fungicide tests. Phytopathology 42: 353-354.

[6] Dr. H. L. Keil, Rohm and Haas Co., Philadelphia, Pa., has reported in private communication that applications of BEBD caused leaf injury on McIntosh and Rome varieties.

[7] The author wishes to thank Mrs. Bessie M. Kennedy for performing these tests, and Dr. Philip Garman for suggestions.

[8] Stoddard, E. M., G. A. Gries, and G. H. Plumb. 1945. Red spider control with disodium ethylene bisdithiocarbamate. Phytopathology 35: 657.

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REPORTS AND ABSTRACTS OF THE ELEVENTH ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The eleventh annual meeting of the Potomac Division of the American Phytopathological Society was held in the Plant Industry Auditorium at Beltsville, Maryland, March 4 and 5, 1954. Approximately 125 persons attended.

Twenty-one scientific papers were presented the first day. The annual banquet was held in the Plant Industry Station cafeteria. Following the banquet, Dr. B. M. Duggar spoke on the "Past, Present, and Future of Plant Pathology." During the second day a symposium on "Antibiotics and the Control of Plant Diseases" was held with Dr. W. J. Zaumeyer as chairman. The topics and speakers were: 1) "Techniques for finding antibiotic substances and potential sources of such materials"—B. M. Duggar; 2) "Chemistry of antibiotics active against plant diseases"—J. R. Garber; 3) "Results with antibiotics in the field and their potential use on additional plants of economic importance"—F. C. Visor; 4) "Food and Drug Administration's position on the use of antibiotics on crop plants" —Henry Welch.

The following officers were elected to serve during the 1955 meeting: *President*, J. W. Heuberger; *Vice President*, H. Rex Thomas; *Secretary-Treasurer*, L. O. Weaver.

H. Rex Thomas, *Secretary-Treasurer.*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Studies on Puccinia asparagi and its relation to Allium spp. Louis Beraha. Teliospores of *Puccinia asparagi* germinated under Illinois conditions immediately after their formation. Infections were obtained on *Asparagus scaber*, *A. acutifolius*, *A. maritimus* and all commercial varieties of *A. officinalis* tested, when they were inoculated with basidiospores. No infections were obtained on *A. scandens* var. *deflexus*, *A. sprengeri*, *A. asparagoides* and *A. plumosus*. Single-pustule urediospore cultures from widely separated areas in Illinois consistently produced a type 4 rust reaction (cereal rust scale) on all susceptible species and varieties. No intermediate types were noted. Urediospores from the same collections were used to inoculate *Allium cepa*, *A. fistulosum*, *A. ascalonicum*, *A. shoenoprasum* and progenies of *A. cepa* × *A. fistulosum* and *A. ascalonicum* × *A. fistulosum* when the plants were in the 6–8 leaf stage. Minute, sparsely sporulating uredia circumscribed by oval chlorotic patches appeared on either the upper or the lower surface of the leaf 8–14 days after inoculation. Older leaves were more susceptible than younger leaves. The reaction obtained consistently resembled the 0 or 1 types. No infection was obtained on *Allium porrum*, *A. sativum* or *A. ampeloprasum*. The uniform but distinct types of reaction obtained on asparagus and onion support the contention that physiological races of the rust fungus, if present, do not account for the loss of supposed resistance in the Washington strains of asparagus.

Factors associated with root-knot nematode resistance in soybeans. H. W. Crittenden. In the search for soybean varieties having resistance to one of the root-knot nematode species, namely, *Meloidogyne incognita* var. *acrita*, it has been found that certain morphological and physiological features appear to be associated with resistance. These features, listed below, may aid in the future search for resistant varieties. The varieties should have some or all of these features: 1) long, tapering roots that penetrate deep into the soil and possess a minimum of lateral roots; 2) roots that have a tendency to become more woody than fleshy early in the development of the plant; 3) acceptable growth and yield in soils containing low amounts of potassium; 4) lowest per cent of oil in the seeds that is commercially acceptable. The variety Laredo has the greatest resistance of any tested to date, but is suitable only for hay or a green manure crop in Delaware. Three commercial varieties (Blackhawk, Monroe, and Anderson), acceptable for seed production in Delaware, appear to have a high degree of resistance to *Meliodogyne incognita* var. *acrita*.

An experiment in growing Merulius lacrymans and Poria incrassata under root-cellar conditions. J. D. Diller and S. W. Huang. The 2 house-decay fungi *Merulius lacrymans* and *Poria incrassata* occur infrequently, but are extremely destructive when they occur in buildings. Unlike other wood-decay fungi, they can start in moist soil and extend many feet into wood that would normally be too dry to decay. An experiment was initiated to determine the advance of the fungi in bolted 2 in. by 4 in. by 8 ft. paired members of oak (*Quercus alba* or *Q. rubra*), pine (*Pinus taeda*), and Douglas-fir (*Pseudotsuga menziesii*), set vertically in a root cellar in pails with infected soil; and to determine the natural resistance of the wood of these species to the 2 fungi. During the 2½ years, the 2 fungi advanced at about the same rate, regardless of wood species, although infection started later in Douglas-fir than in oak and pine. None of the infections has yet reached a height of 8 ft. Both fungi appear to be more active during the spring and summer and less active during the fall and winter.

Experience in growing Merulius lacrymans and Poria incrassata under forest conditions. J. D. Diller and E. J. Koch. The 2 water-conducting, soil-inhabiting, house-decay fungi *Merulius lacrymans* and *Poria incrassata* have rarely been found in specimens sent in for identification and have seldom been successfully grown out of doors. The senior author has recently been successful in establishing 100 inoculants of each fungus in a small wooded area. He used leaves and asphalt-saturated felt paper as insulating materials to maintain high relative humidity and favorable temperatures. During dry periods he supplied water. The authors have obtained weights and some specific gravity readings on 2 in. × 8 in. wedges of 40 durable wood species and have randomized the wedges in these inoculants to test natural resistance. For 8 species, wedges of heartwood, sapwood and half heartwood-half sapwood were included. After six months, ¼ of the wedges were removed and it was found that decay as measured by the percentage weight loss arranged from 0 to more than 50 per cent for the various species.

Identification of the Tranzschelia rust attacking capulin cherry. John C. Dunegan. The species of *Tranzschelia* on capulin cherry leaves collected in South America could

not be identified because of the absence of teliospores. Laboratory inoculations at Beltsville, Maryland, with living material from Ecuador, imported under Quarantine permit, indicated that the rust was *T. pruni-spinosae* since infections developed on *Prunus serotina*, the normal host of *T. pruni-spinosae*, and not on *P. persica*, the host of *T. discolor*. In 1953 this determination was confirmed by a collection of rust showing numerous *T. pruni-spinosae* teliospores on leaves of introduced capulin cherry trees at Fort Valley, Georgia.

Pathogenicity of a spiral nematode (Heliocotylenchus sp.) attacking boxwood. A. Morgan Golden. An ectoparasitic nematode, an undescribed species of *Helicotylenchus* Steiner 1945, has been found almost consistently in large numbers associated with boxwood plants which show a lack of vigor, excessive bronzing of leaves, and a generally unhealthy appearance during hot or dry weather. Surveys have shown this form to be widely distributed on this host in Maryland and surrounding states. A test was conducted in the greenhouse at the Plant Industry Station, Beltsville, Maryland, to determine the effect of this nematode on boxwood and at the same time observe its population build-up in the presence and in the absence of boxwood plants. From the data obtained, it seems evident that this nematode is a damaging parasite, causing a significant reduction in the average root weight of the inoculated plants; that its build-up is quite rapid, increasing from 250 specimens used for inoculation to an average of 7300 per pot in 8 months; and that in soil alone it would not persist indefinitely, the average number per pot being reduced from 250 to 2 in the same period of time. It appears that this nematode, by stunting the root system and weakening the plant, is an important factor in the unhealthy condition of many boxwood plantings.

Pathogenic races of Diplocarpon rosae, the rose blackspot fungus. W. R. Jenkins. Varietal differences in susceptibility of roses to blackspot, caused by *Diplocarpon rosae*, have been noted. There also appears to be variation in the pathogen since the same rose variety grown in different areas showed differences in susceptibility. During 1953, 22 isolates of *D. rosae* from 17 locations in the United States were used to inoculate detached leaflets from a group of 20 roses of the major genetic classes. Inoculum consisted of a suspension of spores washed from the surface of cultures of the fungus grown on canned green pea infusion agar for 5–7 weeks at 70–72° F. Detached leaflets from which the waxy bloom was removed by rubbing were inoculated with a single drop of spore suspension adjusted to contain 2–2.5 $\times$ 10^5 spores per ml. The leaflets were kept on thin cotton mats moistened with a 2 per cent sucrose solution in closed six-inch culture dishes and incubated for 8 days at 75°. They received light about 8 hours a day. Based on the number of test roses infected and diameter of spots after 8 days, 19 distinct pathogenic races were identified among the 22 isolates. Range in pathogenicity was wide, 1 isolate infecting only 8 of the test roses and 3 isolates attacking all 20.

The relative effect of rate of drying and potassium phosphate in the inoculum on plant virus Infections. Robert P. Kahn and Norbert D. Schachtner. The relative effects of the rate of drying inoculated leaves and the presence of potassium phosphate in the inoculum on local lesion production by 4 viruses on bean and/or cowpea were investigated. Inocula were prepared by extracting desiccated tissue with distilled water and with 0.1 M potassium phosphate buffer at pH 7 (phosphate). Celite-dusted primary leaves were inoculated by rubbing with a ground-glass spatula dipped in the inoculum. Tobacco-necrosis virus and tobacco-ringspot virus on cowpeas (var. Black) caused 2-3 times more infection when the leaves were dried quickly than when dried slowly whether or not phosphate was present in the inoculum. This effect of drying was evident for tobacco-mosaic, tobacco-necrosis, and southern-bean-mosaic viruses on bean (Early Golden Cluster and/or Pinto) but only in the absence of phosphate in the inoculum. These observations were based on counts of 500 bean leaves and 280 cowpea leaves distributed in 7-8 trials for each virus. This extends Yarwood's observations on the rate of drying for tobacco-mosaic, tomato-spotted wilt, and tobacco-necrosis viruses in water inocula only as determined from a small number of inoculated leaves.

Conditions influencing Thielaviopsis root rot of poinsettias. John R. Keller. Poinsettia cuttings were grown in soil inoculated with isolates of, *Thielaviopsis basicola, Rhizoctonia sp.*, and *Pythium sp.* to determine how much root rot each caused alone and in combination. After 60 days, roots and tops were weighed. Better than 1 per cent significance in decrease of top weight resulted where root rot was produced by *Thielaviopsis* alone or in combination with *Rhizoctonia* or *Pythium* but none was obtained with *Rhizoctonia* or *Pythium*, or both. Four isolates of *Thielaviopsis* were tested against 11 varieties of poinsettias and 1 of tobacco at 3 greenhouse temperatures and 3 pH levels. *Thielaviopsis* isolates were obtained from poinsettias from Maryland and Ithaca, New York and a brown and a gray strain from tobacco. Excellent root rot control at 50, 60, 70° F. was obtained in acid soil (pH 4.6—4.9). At 70° F., rot development was delayed until the plants neared maturity. At pH levels of 6.2 and 7.2 *Thielaviopsis* from Maryland poinsettias was very pathogenic to poinsettias whereas the Ithaca isolate was only moderately so. Neither isolate caused root rot of tobacco. The brown tobacco isolate injured tobacco but not poinsettias. The gray isolate did no damage to either. Based on pathogenicity, it appears that distinct *Thielaviopsis* strains may exist.

Root galling associated with dagger nematode, Xiphinema diversicaudatum (Micoletsky, 1927) Thorne, 1939. A. F. Schindler. In a survey of nematode diseases of greenhouse grown roses in northeastern U. S., it was found that galling of the rose roots was associated with infections of the dagger nematode, *Xiphinema diversicaudatum*, and not due to root-knot nematodes, *Meloidogyne* spp. This galling is characteristically an enlargement of the tip and curling of the end of the root with an apparent necrosis and shriveling of the proximal portion. This "curly-tip" effect appears to be typical and may be recognized with experience. By inoculating a rose cutting with 945 dagger nematodes, these galls were produced experimentally on the roots. Two controls, 1 of which was a cutting inoculated with water in which the nematodes were collected, the other an untreated cutting, developed no galls on the roots. Galls or curly-tips have also been produced on the roots of seedlings of tomato, soybean, okra, cucumber, balsam, and peanut grown in *X. diversicaudatum* infested soil. In soybean, these nematodes were observed and photographed in the process of feeding upon the roots.

Antibiotic and other chemical dips reduce discoloration of packaged cole slaw. W. L. Smith, Jr. and R. E. Hardenburg. Salability of unrefrigerated cole slaw is limited because it usually darkens appreciably in 1 day. Tests to reduce discoloration consisted of dipping freshly cut slaw in chemical solutions for 20 or 60 seconds and storing it in ventilated cellophane packages for 3 days at 70° F. Slaw dipped in water was barely salable for 1 day, unsalable in 2 because of discoloration, and often decayed in 2 or 3. Only 3 of 95 chemicals tested consistently reduced discoloration. Slaw treated with 0.1 per cent streptomycin sulfate was salable 3 days, with 1 per cent $NaHCO_3$ 2 days, and with 1 per cent NaCl more than 1 day. Reduction of discoloration by combining $NaHCO_3$ or $NaHSO_3$ with streptomycin equaled or exceeded that by streptomycin alone. Bacterial soft rot was consistently controlled only by streptomycin treatments. Slaw in non-ventilated check packages was negligibly discolored in 2 or 3 days but often showed breakdown and had off-odors. Streptomycin treated slaw in non-ventilated packages did not discolor or break down and had little or no off-odor. Crude streptomycin also reduced discoloration and controlled decay.

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Terramycin and aureomycin (0.1 per cent) controlled decay, but caused severe injury.

Post-harvest treatments for the reduction of peach decays. W. L. Smith, Jr., T. T. McClure, and M. H. Haller. Fungicides were applied to hard to firm peaches within 3 hours of inoculation with spore suspensions of *Monilinia fructicola* or *Rhizopus nigricans.* Decayed fruits were counted after 6 days at 75° F. Isothan Q 15, captan, and Dowicide A sprays and sulfur dust reduced *Monilinia* decay 74, 73, 63 and 65 per cent (3-year average). Imidazoline, Shell XP 63, and N 521 sprays reduced this decay 60, 55, and 45 per cent (2-year average). Shell AL 126 and rimocidin, tested as sprays for 1 year, reduced *Monilinia* decay 57 and 44 per cent. Only Dowicide A and rimocidin were effective against *Rhizopus* decay, reducing it 38 and 62 per cent. When fumigation was done at 75° neither tetrachloroethylene nor trichloroethylene was effective against *Monilinia* decay, but both reduced *Rhizopus* decay. Decay control was essentially the same whether volatilization was with heat or without it. Dusting with sulfur followed by tetrachloroethylene fumigation at 75° reduced both decays. When peaches were fumigated at 40°, held 4 days at 40°, and then transferred to 75° neither tetrachloroethylene nor trichloroethylene was effective against either decay. Sulfur dust under the same conditions reduced *Monilinia* decay as much as it did when the peaches were held continuously at 75°.

A device for recording the duration of dew deposits. Carlton F. Taylor. The device consists of an 8-inch ground glass disc mounted on a turntable and rotated by a clock mechanism, and a purple Mongol copying pencil mounted loosely in a vertical guide with the point resting on the disc. A faint mark is left as the dry surface moves past the pencil point but as dew is deposited the pencil becomes moist and the mark becomes broad and dark. Sandblasted glass discs were the best deposition surfaces tested and can be cleaned with acetone. Dew is deposited and dries at approximately the same time that it does on horizontally exposed leaves of most plants. The actual time of dew deposition and its duration are determined by placing the discs over a 24-hour circular recording chart. The length of the line indicating the duration of the dew deposit is accurate to within a few minutes. While the width of the line constitutes an indication of the amount of dew present, the progressive shortening of the pencil in contact with the wet surfaces results in an increasingly heavy mark. The occurrence of rain is indicated by a feathered appearance of the line.

Morning-glory, an indicator plant for the feathery-mottle virus of sweetpotato. R. E. Webb. Several horticultural varieties of morning-glory, *Ipomaea purpurea,* were mechanically inoculated with feathery-mottle-infected sweetpotato leaves. Carborundum was used as an abrasive. The variety Crimson Rambler was the most susceptible to infection and produced the most distinctive symptoms. Fourteen to 18 days after inoculation, small chlorotic spots appeared on the leaves; these were closely followed by slight vein clearing and severe veinal necrosis of many lateral veins, usually extending a short distance into the leaf parenchyma. As the leaf aged, the necrotic tissue cracked and portions dropped out, resulting in a tattered and slightly chlorotic leaf. The more severely affected leaves abscised. Infected plants did not show severe stunting. Systemic symptoms were similar to those produced on the parts initially inoculated. As the infected plants grew, the young leaves, ⅓ to ½ grown, developed a slight mottle closely followed by vein clearing and veinal necrosis. Sap from virus-infected leaves of Crimson Rambler morning glory and sweetpotato plants produced characteristic symptoms of the feathery-mottle virus on mechanically inoculated Porto Rico sweetpotato plants. Several transfers of the virus from plant to plant of Crimson Rambler gave similar results. Healthy scions of Crimson Rambler cleft-grafted to virus-infected Crimson Rambler and sweetpotato plants developed identical symptoms.

Some variations in symptomatology of potato leafroll. R. E. Webb and E. S. Schultz. Leafroll symptoms in different potato varieties are considered to be similar and to vary only in severity. In contrast, current-season symptoms of leafroll in potato seedling X927-3 resemble infection by some yellows viruses. Apical leaves were severely dwarfed and chlorotic with little or no leafroll. Internodes were shortened, giving the apical portion of infected plants a rigid bushy appearance. Late current-season symptoms show a generally chlorotic, apically dwarfed, bushy plant, with many axillary shoots and slight leafrolling. Second-generation symptoms of leafroll in X927-3 were of 3 distinct types. Severe spindle-sprout was the predominant type. The weak sprouts emerged during late July and made little growth. Foliar symptoms of leafroll in the spindle-sprout plants were leaf chlorosis, some anthocyanin development, and leafroll. The second most prevalent symptom was similar to those induced by current-season infection except for the earlier appearance of leaf chlorosis and dwarfing. Infected plants were severely dwarfed, chlorotic, and bushy without leafroll. Plants showing the third type of symptom manifested typical leafroll; they were stunted and chlorotic with a uniform distribution of leafrolling. Tuber production was directly proportional to severity of dwarfing. Leafroll in plants showing the 3 types was confirmed by transferring the virus to *Physalis floridana* Rybd. by *Myzus persicae* (Sulz).